THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME IX, SUPPLEMENT 1933 WHOLE NO. 54

FRANK BURR MALLORY AND THE PATHOLOGICAL DEPARTMENT OF THE BOSTON CITY HOSPITAL *

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The history of a successful institution is often the history of a man. This is particularly true of the pathological department of the Boston City Hospital.

The hospital was dedicated and opened in 1864 for the treatment of acute and chronic diseases. Dr. Charles E. Swan is referred to as pathologist in connection with the dedicatory exercises, but is not mentioned in the annual report for 1864. Like many of his successors Dr. Swan evidently used the position as a stepping-stone to appointment on the clinical staff, becoming physician to outpatients in 1868. Dr. S. G. Webber was appointed pathologist in 1870 and Dr. William P. Bowles in 1873.

The dead house and autopsy room were located on the east side of Albany Street in a part of the boiler house, near the location of the new Mallory Institute. Complaint was made in 1870, "now that Albany Street has become so great and important a thoroughfare," that the carrying of bodies across the street, not at first objectionable, had become so. In 1871 improvements were made in the boiler house and a morgue was established, not only for those dying in the hospital, but also "for the reception and identification of the unknown dead found elsewhere." Record is especially made in 1878 of the appointment of Dr. E. G. Cutler as pathologist.

In 1880 it is interesting to read that "through the liberality of the City Government the Superintendent's office at the hospital has been connected by telephone with police headquarters."

In 1881 Dr. W. W. Gannett was appointed pathologist with

* Received for publication August 23, 1933.

Dr. Cutler, who resigned during the next year. Dr. Gannett remained with the institution as pathologist up to 1891. Although he was the most permanent of the pathologists up to this time, he carried on in addition an extensive private and consulting practice, together with teaching.

In the meantime, as the hospital population grew and the number of deaths increased, the necessity of carrying bodies across Albany Street became so objectionable that the morgue and autopsy room were moved to the main hospital grounds. The autopsy room was located on the second floor of the laundry building. In the early days Louis A. Pasco, who served the department as keeper of the mortuary for forty years before retiring, was also employed in the laundry. One of my earliest memories of the hospital is the picture of Pasco carrying a body on his shoulder up the long flight of stairs to the autopsy room. When the autopsy began Pasco's principal duty was to wave a newspaper or fan to keep the flies from Dr. Gannett's bald head.

It is soberly recorded in the report for 1886, after a long discussion, that women medical students were to be admitted to the amphitheater of the hospital, and a petition that homeopathic treatment might be furnished for those who preferred it was denied.

With the establishment of the South (contagious) department there was needed a laboratory for the diagnosis of diphtheria, and a long narrow room was supplied on the basement floor of one of the wards looking out on the gardener's quarters.

Following the resignation of Dr. Gannett, Dr. Henry F. Sears (donor of the Sears laboratory to the Harvard Medical School), who had been serving as assistant pathologist, was appointed his successor. Within the year Dr. Sears resigned and Dr. William T. Councilman, who had just been elected Shattuck Professor of Pathology at the Harvard Medical School, became pathologist and Dr. Mallory was appointed assistant to the pathologist. The primitive quarters assigned to the pathological department shocked Dr. Councilman, who came fresh from Johns Hopkins University, where pathology was given proper consideration. As the result of his efforts the trustees of the hospital prepared plans and the building occupied by the department up to now was constructed. Dr. Councilman served the hospital as pathologist up to 1911, when he resigned and was made consulting pathologist.

In 1892 Dr. William R. Stokes of Baltimore was appointed resident assistant pathologist. In 1895 Dr. James Homer Wright came from Baltimore to become second assistant to the pathologist. In 1896, when the Massachusetts General Hospital had constructed a building for the department at the behest of Dr. Councilman, Dr. Wright was appointed pathologist to that hospital. The writer became resident assistant pathologist in 1895, *vice* Dr. Stokes, resigned, and had the honor of serving in that capacity when the new building was opened in 1896.

The history of the pathological department, modernized and dignified by the efforts of Drs. Councilman and Mallory, is from this point largely a record of the work of Dr. Mallory.

Frank Burr Mallory came from Cleveland, Ohio, graduated from Harvard College with the degree of A.B. in 1886, and from the Harvard Medical School in 1890, with the degrees of A.M. and M.D. In 1891 he was appointed assistant to the pathologist at the Boston City Hospital and also assistant in histology at the Harvard Medical School. In 1892 he was made assistant in pathology at the medical school, meantime continuing as assistant to the pathologist at the hospital. The year 1893–94 was spent with Chiari in Prague and with Ziegler in Freiburg. On his return he resumed his former position at the hospital but was advanced to the position of instructor at the school. The hospital made him first assistant pathologist the following year and in 1896 he was appointed assistant professor at the school. In 1897 his title at the hospital was changed to assistant visiting pathologist and in 1900 to first assistant visiting pathologist. He was advanced to an associate professorship at the school in 1901, which position he held until 1919. In 1908 he became pathologist to the hospital and served in that capacity until his official retirement last year at the age of seventy. He was then made consulting pathologist. In 1928 he was appointed professor of pathology at the school and held this position until he retired.

During the years since his first appointment as assistant to the pathologist at the Boston City Hospital his activities have centered about that institution.

His publications, alone or with his colleagues or assistants, have covered a wide field. Beginning with a paper entitled "Sacro-Coccygeal Dimples, Sinuses and Cysts" during his first year as assistant in pathology, they include studies on typhoid, diphtheria,

pertussis, scarlet fever, measles, infectious lesions of blood vessels and the classification of nephritis.

His principal interests were reflected in a series of papers on improvements in histological technique beginning in 1895, which involved the working out of new staining methods that have carried his name to all quarters of the world. Particular attention was given to collagen (reticulum), neuroglia, myoglia and elastica, and a new element, the fibroglia fibril, was described in 1903. A collection of papers published from 1902 to 1920 dealt with the application of special histological methods to the study of tumors, with particular reference to the embryological origin of each type of cell entering into newgrowths, and the differentiation that each type undergoes. Mallory's studies in histological methods served to revolutionize the crude procedures in vogue, particularly in American laboratories, and gave a new impetus to the exact study and classification of tumors.

With a preliminary paper on necroses of the liver in 1901, and a second in 1911 on five types of lesions that may give rise to cirrhosis of the liver (which represented the carefully digested results of years of observation, and included demonstration of the hyaline change characteristic of alcoholic cirrhosis), Dr. Mallory's attention tended to focus itself more and more on diseases of the liver. When exact observation and the most careful histological technique could add little more to our knowledge of the cirrhoses, resort was had to experimentation. These studies resulted in the demonstration that the lesions of pigment cirrhosis could be reproduced by the ingestion of copper, and the finding of copper in distilled liquors explained the relation that was known to exist between pigment cirrhosis and alcoholism. Hemochromatosis ceased to be a mystery. The alcoholic form of cirrhosis was a harder problem to solve. For over twenty years practically continuous efforts to produce this disease in a great variety of experimental animals have been carried on. Alcohol alone, the metals and any other agencies that suggested themselves, have been tried out without result. Recent work, however, indicates a successful end to this long search. Phosphorus, outstanding above all known substances, appears to show a highly selective poisoning effect on liver cells, even in infinitesimal doses, and a typical cirrhosis of alcoholic character has been produced in experimental animals.

One of the characteristics of Mallory publications, in addition to clearness of exposition, has been the insistence upon adequate illustration. From his first paper onward the quality of the drawings and photomicrographs has been the highest. Color was first used in a series of drawings illustrating a study of the staining reactions of neuroglia, in a paper in German in the *Centralblatt für allgemeine Pathologie und pathologische Anatomie* in 1895. In 1898 came the paper on the histology of typhoid, with its beautiful colored plates, which set a high standard for medical illustrations. In the more recent period, as he perfected and taught the technique of photomicrography, black and white reproductions, whose scientific exactness could not be questioned, tended to replace color. As a teacher and an editor he has insisted that high quality of illustrations is a prime essential for medical publications, and has helped to lift the level of American medical illustration to its present high point.

It is of interest that Dr. Mallory in 1906, in a paper, "The Present Needs of the Harvard Medical School," presented, not the needs of the laboratory branches, but the pressing want of endowment for clinical departments, with salaries large enough to permit clinical teachers to give up all of their time to the work.

Dr. Mallory, with Dr. J. Homer Wright, published first in 1897 "Pathological Technique," which became the laboratory bible for a generation, and embodied the principles laid down by Mallory with reference to clearness of exposition, conciseness and adequate illustration. In 1914 Dr. Mallory published "The Principles of Pathologic Histology."

In addition to the publications mentioned Dr. Mallory served from 1923 as editor-in-chief of the *Journal of Medical Research.* When the name of this journal was changed to *American Journal of Pathology* in 1925, he continued as editor-in-chief, in which position he is still as active as ever. He has also served as treasurer of the American Association of Pathologists and Bacteriologists since 1911. He is a member of the Commission on Standardization of Biological Stains, a corresponding member of the Royal Medical Society of Budapest, and an honorary member of the Philadelphia Pathological Society. He holds the honorary degree of Sc.D. from Tufts College, and a similar degree from Boston University.

More remarkable, however, than the production of publications has been the production of men. When the pathological department

of the Boston City Hospital was moved from its single room into the building now occupied, there was opportunity for expansion of the service, particularly in the direction of postgraduate teaching. Starting with a resident assistant pathologist and a single interne the staff has grown to include an assistant director, four assistants and five internes. Through this laboratory during the years has passed a notable group of young men, destined to attain distinction in the teaching of medicine. The stay in the laboratory has been limited, with few exceptions, to a period of three years or less. Each neophyte was required to progress through a series of assigned duties from the sharpening of knives, the practice of histological technique, bacteriological diagnosis, daily conferences on surgical diagnosis, autopsy technique with detailed reports on the gross and microscopic findings, to methods of teaching and of research. The work was laid out in such manner that the graduate from the department could step into a laboratory equipped to oversee every function of technician and diagnostician, and with a foundation that fitted him to carry out research, to teach the subject of pathology or apply its principles to the practice of medicine or surgery.

If he determined to enter one of the clinical medical branches as his life's work, he found himself prepared to visualize clearly the changes that the organs of his patients had undergone, and was enabled to apply more intelligently whatever therapy was indicated. If his chosen field were surgery, his training in surgical pathology made him competent to recognize gross pathological lesions as he met them, and adapt his operative procedures to the conditions discovered. If he entered the field of research, he was endowed with a knowledge of the reactions of the animal body that qualified him to understand and interpret the results obtained in his experimental researches.

Moreover his training was overseen personally by Dr. Mallory, with whom in frequent conferences his critical sense was ripened, he was taught the need of scientific accuracy, and a horror of slipshod methods. Constant association with an enthusiastic inquiring spirit served to inspire him with that scientific curiosity — that will to know — which is the *fons et origo* of medical progress.

Following is a list of the graduates of the department in chronological order.

TIMOTHY LEARY. Professor Emeritus of Pathology, Tufts College Medical School. Medical Examiner, Suffolk County, Massachusetts.

*RICHARD M. PEARCE. Formerly Professor of Pathology and Bacteriology, Albany Medical College, and Director, Bender Hygienic Laboratory, Albany, N. Y.; Professor of Pathology, University and Bellevue Hospital Medical College, and Director, Division of Medical Education, Rockefeller Foundation, New York, N. Y.

LAWRENCE W. STRONG. Formerly Pathologist to the New York Eye and Ear Infirmary and the Woman's Hospital, New York, N. Y. Deer Isle, Maine, not in practice.

JOSEPH H. PRATT. Professor of Medicine, Tufts College Medical School. Physician-in-Chief, New England Medical Center, Boston, Mass.

* WILLIAM H. DOWNEY. Formerly in general practice, Peabody, Mass.

WILLIAM H. BARTON. Medicine. Temple, Texas.

D. HAROLD WALKER. Professor Emeritus of Otology, Harvard University Medical School. Visiting Otologist, Children's Hospital, Massachusetts General Hospital, and New England Hospital for Women and Children, Boston, Mass.

GEORGE BURGESS MAGRATH. Professor of Legal Medicine, Harvard University Medical School. Medical Examiner, Suffolk County, Massachusetts.

HARRY C. LOW. Visiting Surgeon, Little Wanderers Home, Boston, and Children's Sunlight Hospital, Egypt, Mass. Formerly Visiting Surgeon, Massachusetts General Hospital, Boston, Mass.

FRANK T. FULTON. Consulting Physician and Cardiologist, Rhode Island Hospital, Providence. Consulting Physician, Providence City Hospital, Pawtucket Memorial Hospital, Woonsocket Hospital, and Westerly Hospital, Rhode Island.

*H. A. STEENSLAND. Formerly Professor of Pathology, Syracuse University College of Medicine, Syracuse, N. Y.

HENRY A. CHRISTIAN. Professor of Medicine, Harvard University Medical School. Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass.

*WALTER R. BRINKERHOFF. Formerly Assistant in Pathology, Harvard University Medical School; Fellow of the Rockefeller Institute for Medical Research, New York, N. Y.; Physician to the Carnegie archeological expedition to the Transcaspian Territories; Director of the Molokai Leprosy Investigation Station of the United States Public Health Service, Hawaiian Islands; Assistant Professor of Pathology, Harvard University Medical School, Boston, Mass.

*ELMER E. SOUTHARD. Formerly Professor of Neuropathology, Harvard University Medical School, and Director of the Boston Psychopathic Hospital, Boston, Mass.

RALPH L. THOMPSON. Professor Emeritus of Pathology, St. Louis University School of Medicine. Director, National Pathological Laboratory, St. Louis, Mo.

RALPH E. LEE. Douglaston, N. Y., not in practice.

NATHANIEL H. GIFFORD. Surgery. Providence, R. I.

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S. Burt Wolbach. Professor of Pathology, Harvard University Medical School. Pathologist, Peter Bent Brigham Hospital, Children's Hospital, and Boston Lying-in Hospital, Boston, Mass.

Charles W. Duval. Professor of Pathology, Tulane University of Louisiana School of Medicine, New Orleans, La.

*Paul A. Lewis. Formerly Assistant Professor of Pathology, University of Pennsylvania School of Medicine; Director, Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.; and Associate Member, Department of Animal Pathology, Rockefeller Institute, Princeton, N. J.

George S. Graham. Professor of Pathology, Graduate School of Medicine of the University of Alabama, Birmingham, Ala.

Samuel T. Orton. Professor of Neurology and Neuropathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Harold E. Robertson. Professor of Pathology, University of Minnesota Medical School, Minneapolis. Pathologist, The Mayo Clinic, Rochester, Minn.

*Oliver R. Mabee. Formerly in the practice of surgery, Toronto, Canada.

Burdett L. Arms. Farmington, Maine. Formerly Bacteriologist, Division of Diagnostic Laboratories, State Board of Health, Jacksonville, Fla.

Lawrence J. Rhea. Associate Professor of Pathology, Faculty of Medicine, McGill University. Pathologist, Montreal General Hospital, Montreal, Canada.

Arthur B. Emmons. Executive Director, American National Red Cross, Boston Metropolitan Chapter, Boston, Mass.

*Freeman D. Bosworth. Formerly in practice, Stockbridge, Mass.

Samuel R. Haythorn. Professor of Preventive Medicine, University of Pittsburgh School of Medicine. Director, William H. Singer Memorial Research Laboratory, Pittsburgh, Pa.

Thomas Ordway. Professor of Medicine and Dean, Albany Medical College, Albany, N. Y.

Archibald W. Hunter. Visiting Surgeon, Vancouver General Hospital. Pathologist to the Coroner, City of Vancouver, Vancouver, B. C.

Harry S. Bernton. Associate Professor of Hygiene and Preventive Medicine, Georgetown University School of Medicine, Washington, D. C.

Oliver S. Hillman. Clinical pathology. Instructor, New York Post-Graduate Medical School and Hospital, New York, N. Y.

Daniel J. Hurley. Surgical Examiner, U. S. Veterans Bureau, Boston. Member, Massachusetts Board of Registration in Medicine, Boston, Mass.

Alexander M. Burgess. Assistant Professor of Biology, Brown University. Physician-in-Chief, Department of Tuberculosis, and Visiting Pathologist, Providence City Hospital, Providence, R. I.

Halsey B. Loder. Consulting Surgeon, Boston City Hospital. Visiting Surgeon, New England Baptist Hospital, New England Deaconess Hospital and Palmer Memorial, Boston, Mass., Newton Hospital, Newton, Mass.

Richard S. Austin. Professor of Pathology, University of Cincinnati College of Medicine. Pathologist, Cincinnati General Hospital, Cincinnati, O.

James S. Tomkies. Pathology. Ottawa, Canada.

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sylvania School of Medicine. Director of Laboratory, Methodist Episcopal Hospital, Philadelphia, Pa.

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FRANK A. MCJUNKIN. Professor of Pathology, Loyola University School of Medicine, Chicago, Ill.

L. WHITTINGTON GORHAM. Professor of Medicine, Albany Medical College, Albany, N. Y.

EDGAR M. MEDLAR. Director, Hegeman Memorial Research Laboratory, Mount McGregor, N. Y.

LLOYD P. MACHAFFIE. Medicine. Ottawa, Canada.

ARCHIBALD H. BEARD. Assistant Professor of Medicine, University of Minnesota Medical School, Minneapolis, Minn.

LEROY U. GARDNER. Director and Pathologist, The Saranac Laboratory for the Study of Tuberculosis, Edward L. Trudeau Foundation, Saranac, N. Y.

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WARD H. COOK. Director, Bureau of Laboratories, Yonkers, N. Y. Pathologist, St. John's Riverside Hospital, Yonkers General Hospital, St. Joseph's Hospital, and Professional Hospital, Yonkers, N. Y.

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WILLIAM S. COCHRAN. Medical Missionary, China.

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CHARLES W. STEELE. Medical Interne, Boston City Hospital, Boston, Mass.

RALPH L. IRWIN. Formerly Visiting Pathologist, Sanatorium Division of the Boston City Hospital, Mattapan, and Pathologist, Cambridge City Hospital, Cambridge, Mass.

WESTON F. SEWALL. Surgical Interne, Boston City Hospital, Boston, Mass.

DAVID F. LOEWEN. Formerly Medical Interne, Presbyterian Hospital, Chicago, Ill.

CLAUDE E. WELCH. Surgical Interne, Massachusetts General Hospital, Boston, Mass.

WILLIAM H. HOLTHAM. Surgical Interne, Boston City Hospital, Boston, Mass.

JOSEPH E. HAMILTON. Medical Interne, Strong Memorial Hospital, Rochester, N. Y.

PAUL R. HINCHEY. Surgical Interne, Boston City Hospital, Boston, Mass.

WILMER C. SMITH. Surgical Interne, Boston City Hospital, Boston, Mass.

In addition to this group of graduates a number of men, including scholars of the National Research Council and the Rockefeller Foundation, have spent varying lengths of time in the laboratory.

As one looks back over the record of these productive years one is inclined to believe that the remarkable results that have been obtained represent more than the activities of a single man. They have more the character of the products of a vital force. And it is a pleasure to report that this vital force, which is Frank Burr Mallory, is still as active, alert and delightfully enthusiastic as it has been for forty odd years. Moreover, the production of men is still being

carried on under Dr. Mallory's kindly oversight as a volunteer, by the man he trained and selected to be his successor, Dr. Frederic Parker, Jr.

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Since the medical examiner service of Suffolk County, Southern district, has been housed with the pathological department of the hospital for years, and its association with the department has become very close, a word about its origin and development is perhaps desirable.

The medicolegal relation of the hospital receives its first notice in the annual report of 1871, with a reference to the establishment of facilities for the reception and identification of the unknown dead found elsewhere.

Up to 1877 Massachusetts had a coroner system inherited, through the colonial and provincial laws, from the Anglo-Saxon practice. Scandals that arose in Suffolk County, which includes Boston, led to the abolishment of the coroner and the replacement of this anachronistic system by the medical examiner system. This change was brought about by the combined action of the Massachusetts Bar Association and the Massachusetts Medical Society. The coroner was called upon to exercise both medical and legal functions with requirements beyond the limitations of most human capacities. Developed in the early years of Anglo-Saxon civilization, when the coroner actually represented the crown in legal affairs, the clumsy inefficiency of the system became manifest as judicial and police systems were developed. When a man dies as the result of violence two basic questions require an answer. The first, "What caused the death?" calls for the exercise of purely medical functions. The second, "Who caused the death?" is a matter for the police and the courts. The new law placed the responsibility of determining the cause and manner of the death upon medical examiners, men presumably selected for their fitness to answer the requirements. The determination of who might be the guilty party, and the degree of his guilt, was left to the police and the courts.

Two medical examiners for Suffolk County were appointed to replace the forty-three coroners who were in office when the law was passed. The county was divided by agreement, the Southern district focussing at the Boston City Hospital. Dr. Frank W. Draper,

who was connected with the staff of the hospital, was appointed medical examiner and served until 1905. Dr. William McDonald, who replaced him, died in 1910. The writer, who had been associate medical examiner since 1908, was then appointed medical examiner and has served since. In 1913 the addition of a second floor to the wing of the pathological building furnished quarters for the medical examiner service.

The kindly coöperation which Dr. Mallory has practised and the close association with his progressive department have helped to bring about whatever success has been achieved by the medical examiner service in this district.

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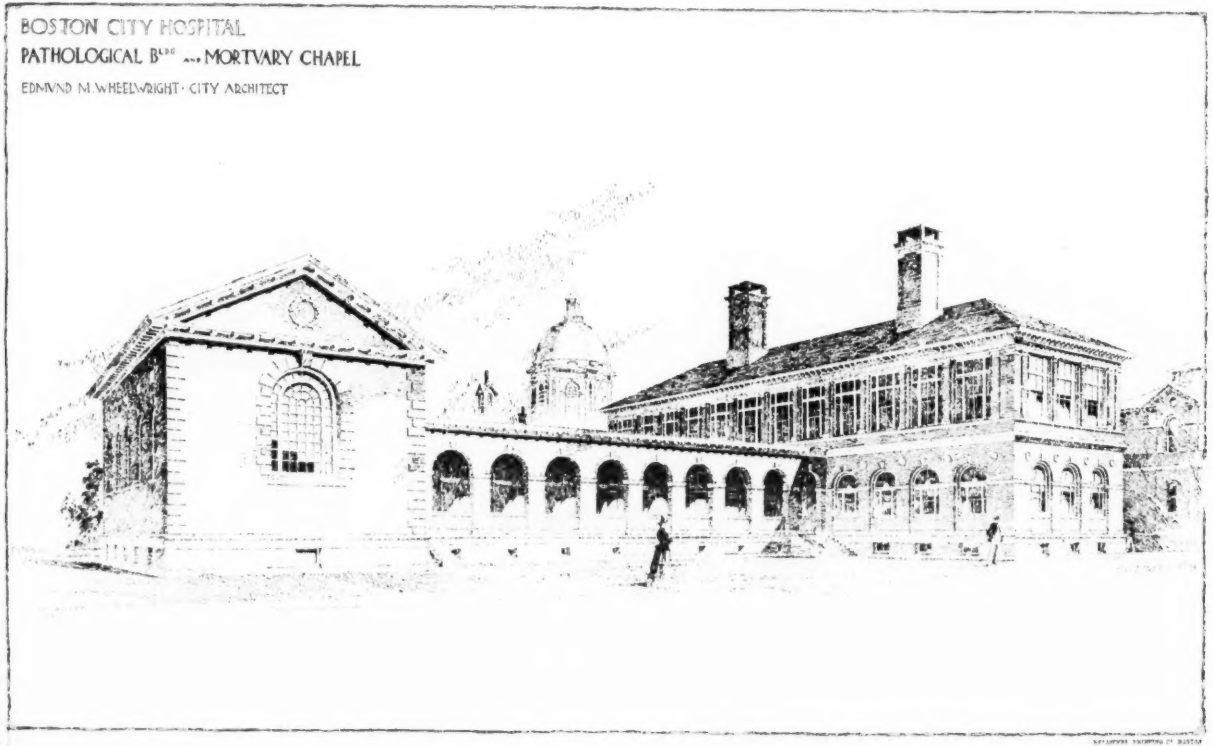
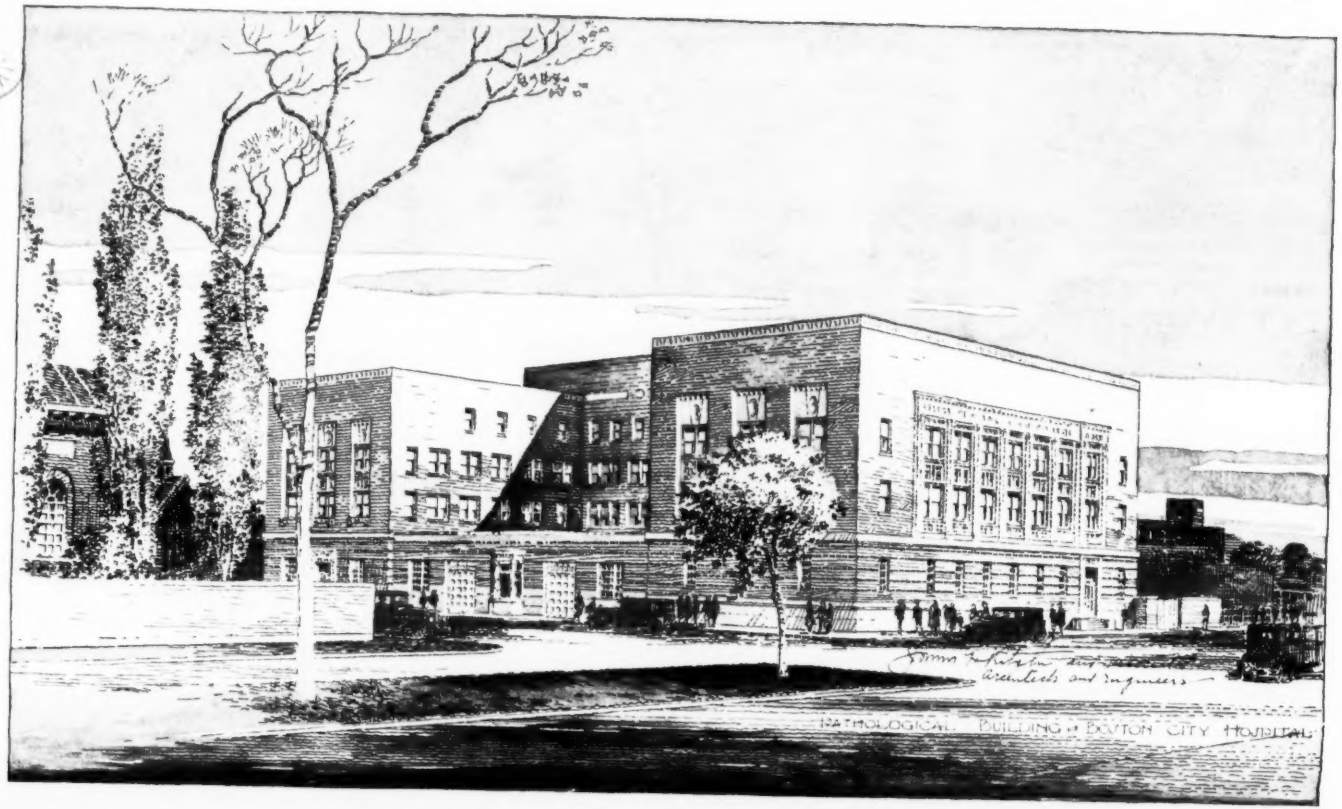
It is fitting that the new building which is to harbor the department that has grown so great under his hands should bear the name of Mallory. The building houses more than the department of pathology, since it will include offices and facilities for the medical examiner service. It is properly named "Mallory Institute of Pathology."



The Original Pathological Building



The Mallory Institute of Pathology of the Boston City Hospital

THE MALLORY INSTITUTE OF PATHOLOGY *

ROBERT N. NYE, M.D.

(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

The old Pathological Laboratory of the Boston City Hospital was formally opened in December, 1895. That the building of this unit was thought by the Trustees to involve a considerable, but fully justified, expense can be gained from their thirty-second annual report, published in 1896: "No modern hospital . . . can be considered as having the proper facilities for doing scientific medical work without a well-equipped laboratory. The Trustees believe that, while the cost of these laboratories has been large, they have exercised wisdom in affording to the professional staff of the hospital every facility for the treatment of patients fully within the most advanced lines of modern scientific medicine." During the next few years the capacity of the hospital was rated at about 800 beds. The staff of the laboratory consisted of a pathologist, two assistant pathologists, two assistants, two pathological internes, four clerical and technical assistants, and in addition the personnel of the medical examiner's office for Suffolk County, South. The routine work of the laboratory averaged, annually, 230 autopsies, 750 surgical specimens, 1100 bacteriological examinations and about 18,000 throat cultures for diphtheria bacilli; the annual expense was about $9000. Teaching was confined to students from Harvard Medical School. Medicolegal cases averaged about 500 a year.

Due to the greatly increased demands placed on the Boston City Hospital and to the obsolescence of many of the buildings, an extensive building program was started about ten years ago. Many of the oldest buildings have been replaced, while others have been modernized. The Trustees plan, eventually, to increase the capacity of the Main Department to well over 2000 beds; at present it is about 1450 beds. These, together with 300 beds at the South Department and 625 beds at the Sanatorium Division at Mattapan, give a total capacity of 2375 beds. The work of the laboratory has increased proportionately. Last year about 700 autopsies were per-

* Received for publication May 15, 1933.

formed and, at the present rate, there will be nearly 800 this year. Approximately 4300 surgical specimens are handled and about 8500 bacteriological examinations made, in addition to 23,000 diphtheria release cultures and 700 pneumococcus typings. About 22,000 Kahn tests are performed annually. Over 1300 cases are certified by the medical examiner or his assistant each year. The laboratory staff consists of a consulting pathologist, one pathologist, two assistant pathologists, three assistants, five pathological residents, seven technicians and one stenographer, and in addition the medical examiner, two assistant medical examiners, one technician and two stenographers. The maintenance of the laboratory proper requires an annual expenditure of about $50,000. Positions in the departments of pathology of Harvard, Tufts, and Boston University Medical Schools are held by members of the staff and a considerable amount of their time is devoted to routine pathological teaching, pathological conferences and postgraduate teaching.

In view of the marked increase in activities it is not surprising that the Trustees, once again, felt obliged to provide adequate laboratory facilities and a new building was included in the expansion program. Land on the easterly corner of Massachusetts Avenue and Albany Street, just across from the Main Department, was purchased in 1931, and in 1932 a sum of $670,000 was appropriated for the building. All the bids on the general contract were surprisingly low and the building was completed at a cost of approximately $460,000, including extras and architects' fees. Ground was broken in August, 1932, and the building was ready for occupancy just about a year later.

The Trustees were most generous in allowing the senior members of the laboratory staff unlimited freedom in planning the interior arrangement and in specifying the fixed equipment; and the architects, James H. Ritchie and Associates, did their utmost to conform to these plans and specifications. The sum of $80,000 was appropriated for furnishing and equipping.

General Plan of Building

The building has a frontage of 111 feet on Massachusetts Avenue and extends back 183 feet. It consists of a basement, four main floors and a penthouse. Above the first floor the building is U-shaped, with

the open end toward Albany Street; the base is 183 feet long and each wing 62 feet long, all being about 50 feet in width. The steel framework rests on deep concrete caissons. The floor and roof slabs are of concrete, conforming to "steel tile" construction, and in general, all partitions consist of gypsum or terra-cotta blocks. The volume of the building is slightly over 1,000,000 cubic feet.

The exterior finish is red brick with cast stone trim. The front is moderately decorated with bas reliefs in cast stone. Between the pilasters and the windows of the second and third floors are seven cast aluminum panels with a design in bas relief, containing, as a central motif, the bishop's cap and rose of the Barber Surgeons of London. These panels are repeated on the ends of the wings, and the spandrels above the third floor windows are ornamental discs containing, as motifs of health, medicine and surgery, respectively, the radiant head of Apollo from the Physicians of London, the caduceus and the medieval lancets from the Barber Surgeons of London.

There are 361 windows, exclusive of the garage. These are located, chiefly, on the second and third floors. Practically all of the animal rooms on the fourth floor are lighted from above by skylights. Electrically and manually operated opaque shades are provided, respectively, in the amphitheater and in the photographic room and museum on the second floor and the conference room. The lower sashes of the larger windows in the autopsy rooms and both sashes of the standard windows in the autopsy rooms, undertakers' rooms and toilets are glazed with Seynite glass.

The floors of the corridors are covered with green asphalt tile, except in the basement and on the fourth floor, where terrazzo or a granolithic finish is used. All laboratory floors are covered with green linoleum and those of the entrance lobby, amphitheater, library and offices with rubber tile. In all toilets, storerooms, autopsy rooms, animal rooms and others that are apt to be wet, terrazzo or granolithic floors are used. The walls, in general, are of plaster on terra-cotta or gypsum blocks and are painted a pale green. In all storerooms and animal rooms, buff paint has been applied directly to the smooth faced terra-cotta blocks. The walls of the mortuaries, undertakers' rooms and small autopsy room on the fourth floor consist of white Ar-ke-tex tile. The ceilings, as a rule, are the painted undersurfaces of the floor slabs. In some instances they are furred down and plastered to cover the service piping, thus improving the

attractiveness of the room, as in the library, offices and entrance lobby. Otherwise, all piping is exposed.

Natural ventilation is used in the majority of the laboratories. In the amphitheater, large autopsy rooms and large conference room, heated air is supplied and exhausted. In the two stock animal wings heated air is supplied in the corridors and drawn through ventilating panels in the doors by exhausts located in each room. In certain other rooms, such as all toilets, the two darkrooms, the undertakers' rooms and the mortuary rooms, air exhausts only are provided. Large hoods connected with power exhaust fans are placed over all sterilizers and gas ranges. All chemical hoods are provided with individual exhaust fans.

The electric lighting fixtures are in part of a type that controls the areas of light distribution. The autopsy tables are lighted by three or five Holophane ceiling boxes, each containing a 150 watt lamp, reflector and lens. This results in an even, shadowless illumination, equivalent photometrically to 100 to 150 foot candles.

The vacuum system of steam heating is used and is controlled in each room automatically. In all of the laboratories with fixed counters or benches the radiators are placed beneath the windows with outlet grills set into narrow shelves at the level of the window ledgers. Celotex fronts on the sheet metal radiator cabinets protect against excessive radiation underneath the counters. As mentioned previously, the amphitheater, two large autopsy rooms and a conference room are heated indirectly. The stock animal wings are heated to about 50° F. indirectly, but each room can be raised to a higher temperature by means of auxiliary radiators, automatically controlled.

Electricity, gas and compressed air are supplied to all the laboratories. At least one complete set of outlets on the counter and one on a small service shelf on the side or back wall are provided in each room. Both the lighting and power lines are 110 volts D.C., but the autopsy rooms and the physiological laboratory are also supplied with 110 volts A.C. for use with a portable roentgen-ray machine. Medium pressure lines furnish steam at 60 pounds for all the chemical hoods, sterilizers and stills. There is an automatic water still and storage tank on the fourth floor and the latter is connected to outlets in the corridors of the second and third floors by means of block tin-lined brass pipe.

There are two elevators — a large service elevator, manually controlled, and a small automatic passenger elevator. A dumb-waiter for gross specimens and heavy glassware extends from the basement to the fourth floor. A rubbish chute goes from the third floor to the basement. All rubbish and refuse on the fourth floor is disposed of in a gas-fired incinerator.

Refrigerating machinery in the basement furnishes brine for the mortuaries, cold rooms and a cooler for the water to the drinking fountains. An electrically heated incubator room is built into the bacteriological suite on the second floor.

Both the fixed and movable equipment have been standardized as much as possible. The counters, or benches, are 30 inches high, 24 inches deep and are set against 6 inch aprons, dropping down from service shelves 12 inches wide, at the level of the window ledges. On the face of this apron are placed the various service outlets, and the heat outlet grills are set flush with the top surfaces of the service shelves. The tops of the counters are made of 4 inch birch strips $1\frac{3}{4}$ inches thick, and have the common black acid-proof finish. Drawers and cupboards, underneath, are placed so that they do not interfere with space in front of windows. An overhead bench light duplicates each window unit.

The majority of sinks are built of alberene stone with drain boards of the same material. They vary considerably in dimensions according to the location. Each is provided, in general, with a lighting fixture, needle valve washing outlets, a removable peg board, and a substantial overhead shelf, on the underside of which are adjustable grooves for holding graduated cylinders.

Nearly all of these cupboards, cabinets and cases are movable. They are constructed of wood and have a unit width of 4 feet. Those with an upper section have a usable height of 7 feet. Tables are 5 or 10 feet long and 30 or 36 inches high; the tops are usually of wood with acid-proof finish, but some are of rubber or of alberene stone.

All the storerooms are equipped with metal shelving of dimensions adapted to the particular use of each room.

Basement

A passenger and service tunnel from the Main Department enters the building in the northeast corner of the basement.

The area under the ambulance garage is unexcavated. A large storage room for autopsy specimens and general supplies occupies practically all of the space under the north wing, and three rooms in the immediate vicinity are required for the refrigeration machinery, main electric switchboard and the air compressors and vacuum pumps of the heating system.

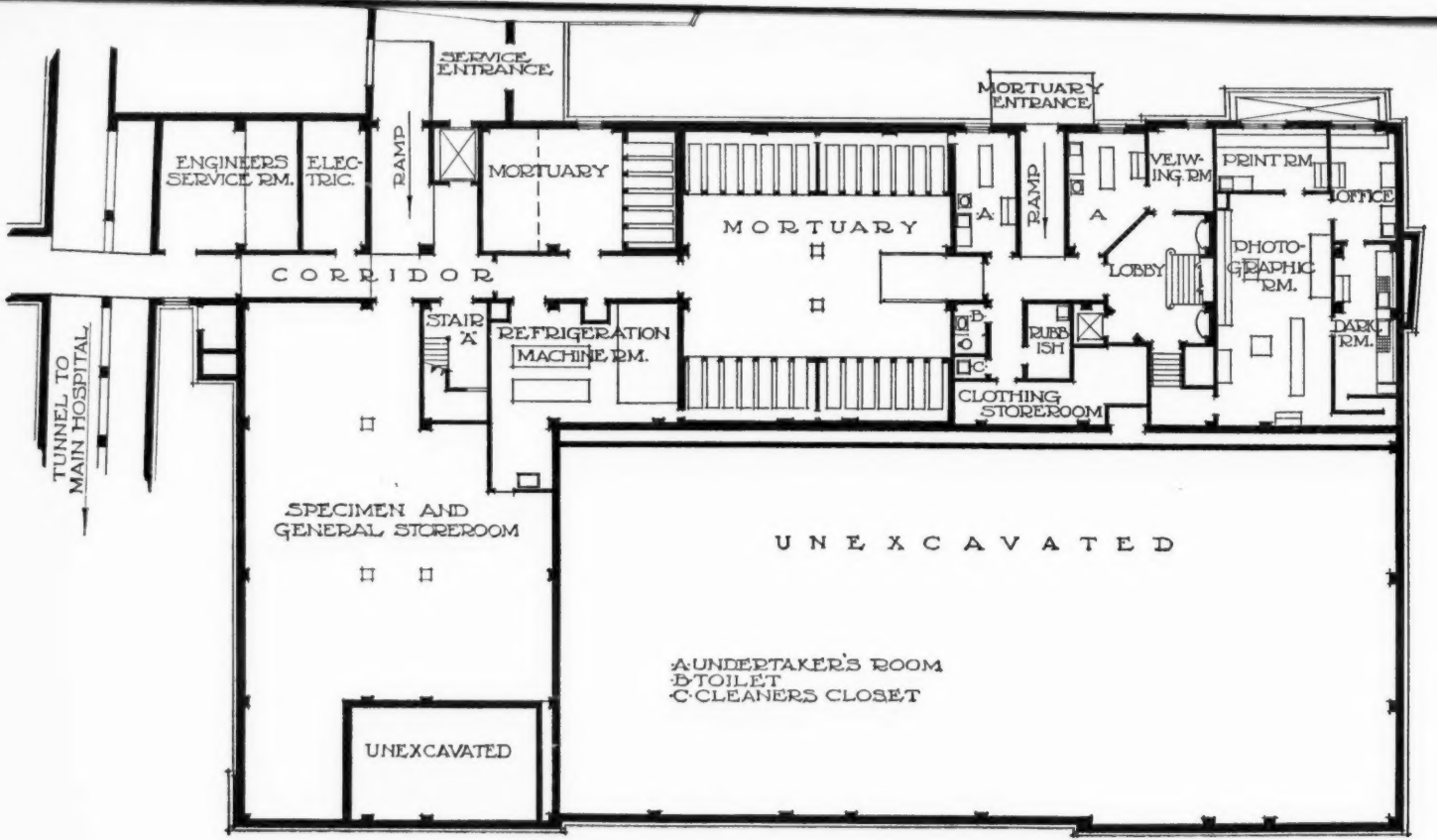
The main mortuary is centrally located, with one entrance from the corridor connecting with the tunnel and another connecting with an outside loading platform on the east side of the building. There are 2 refrigerated compartments, each containing 42 trays and cooled to about 34° F. There is an auxiliary mortuary with 1 box and 17 trays, with provision for an additional box of equal size. This is cooled to about 25° F. and is used for unclaimed bodies and for the unpleasantly odoriferous ones of certain medicolegal cases. At each side of the corridor to the loading platform is a room equipped with a standard embalming table, sink, lavatory and cabinet for the convenience of the undertakers. From one leads a small viewing room, which, in turn, opens into the hall at the foot of a short flight of stairs from the entrance lobby.

The photographic unit occupies the end of the basement and the floor grade is 4 feet lower than the level of the main basement. There is a large inside room completely equipped for photographing charts, and gross and microscopic specimens, for enlarging and reducing, for projecting lantern slides, and for the storage of negatives. A large darkroom with a special sink and a printing booth connects with the inside room by door and by maze and with a small office by another door. The office and a print storage room both receive daylight from an areaway.

First Floor

All the space beneath the south wing and that between the two wings is occupied by an ambulance station. There is no connection between this garage and the rest of the building.

An amphitheater occupies practically all of the north wing, extending up to include the second floor. Entrance is available from



·BASEMENT PLAN·

SCALE 0 5 10 15 20 FEET

the first or second floors of the main building or from a doorway on Albany Street, which connects by a stairway to the back of the amphitheater on the second floor. The amphitheater has a seating capacity of 148. The demonstration bench is arranged with a hidden sink and service outlets, so that the room can be used as a chapel, when necessary. On the front wall are three sections of blackboard and, above, two chart racks. The wall space between the latter serves as a projection screen and the level of the floor of the projection room is such that the midpoint of the horizontal projecting ray is centered in this space. The projection room is built to conform to the requirements of the building code for motion-picture projection. The opaque window shades are controlled by a switch on the front wall, and a buzzer in the projection room is operated by a cord button, similarly located. The amphitheater preparation room is furnished with a sink, lavatory and cabinets, and is of sufficient size for the storage of charts and microprojection apparatus.

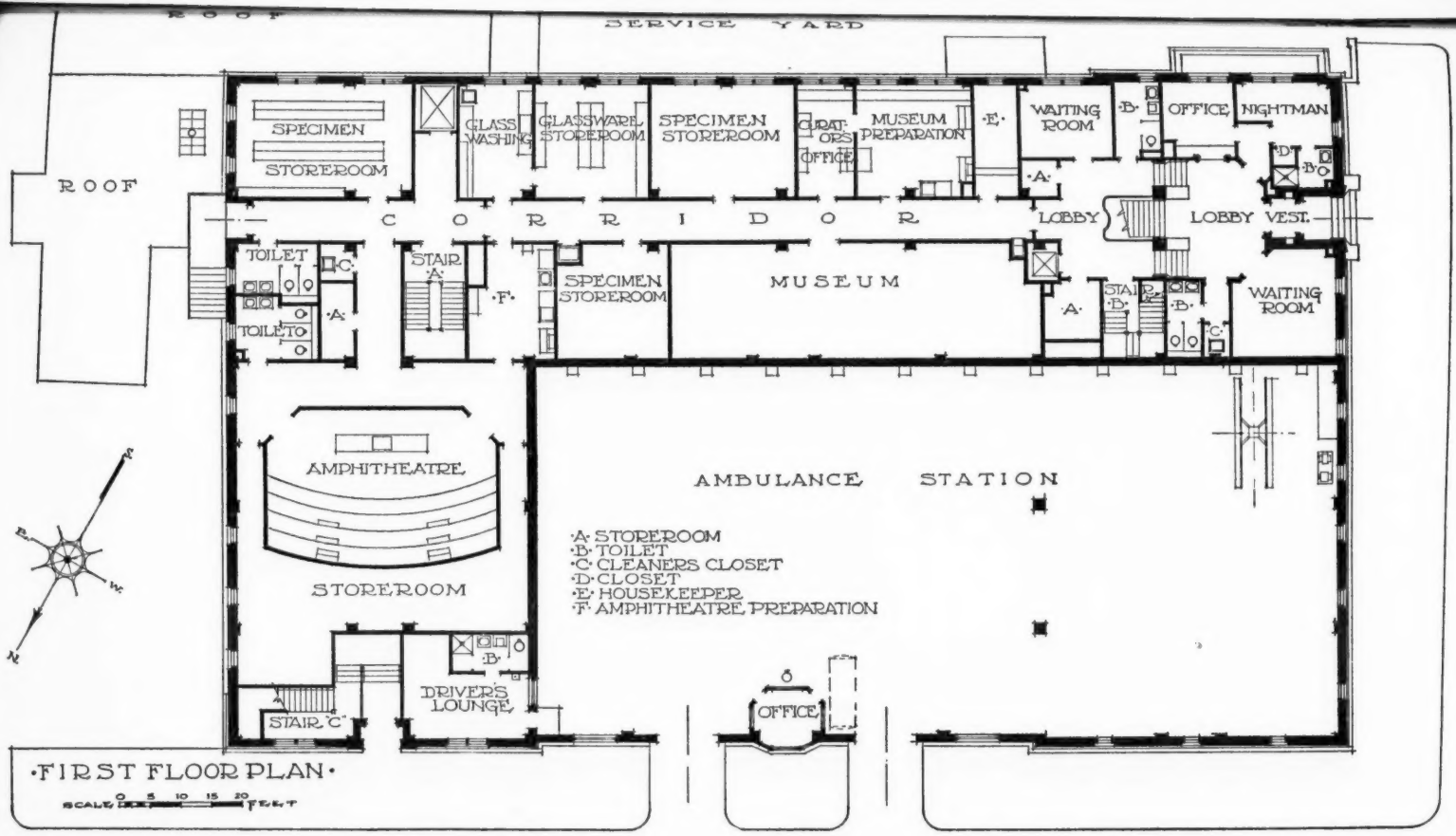
Four rooms are used for storage purposes. One for paraffin blocks has brine coils automatically controlled so that the room temperature never goes above 65° F., even in the hottest weather. Two others are for surgical specimens and a fourth for jars and other heavy glassware. There is a washroom connecting with the latter.

The museum is a room 61 feet long and 19 feet wide and is without windows. The specimens are kept in dust-proof steel museum cases and are adequately lighted by special fixtures. On the opposite side of the hall are an office for the curator and a museum preparation room which, in addition to the standard laboratory equipment, contains a large motor-driven band saw.

The main entrance lobby is at a lower floor level than the remainder of the first floor. Opening from this are a waiting room, an office, and a bedroom and bath for the man on night duty. At the higher level, but still a part of the same unit, are an additional waiting room, a housekeeper's room, small storerooms and toilet facilities.

Second Floor

The north end of the main part of the building is devoted to bacteriology and serology. The suite for routine bacteriology consists of a utility room and a bacteriological laboratory connected by a narrow passageway. On the outer side of this passageway are two



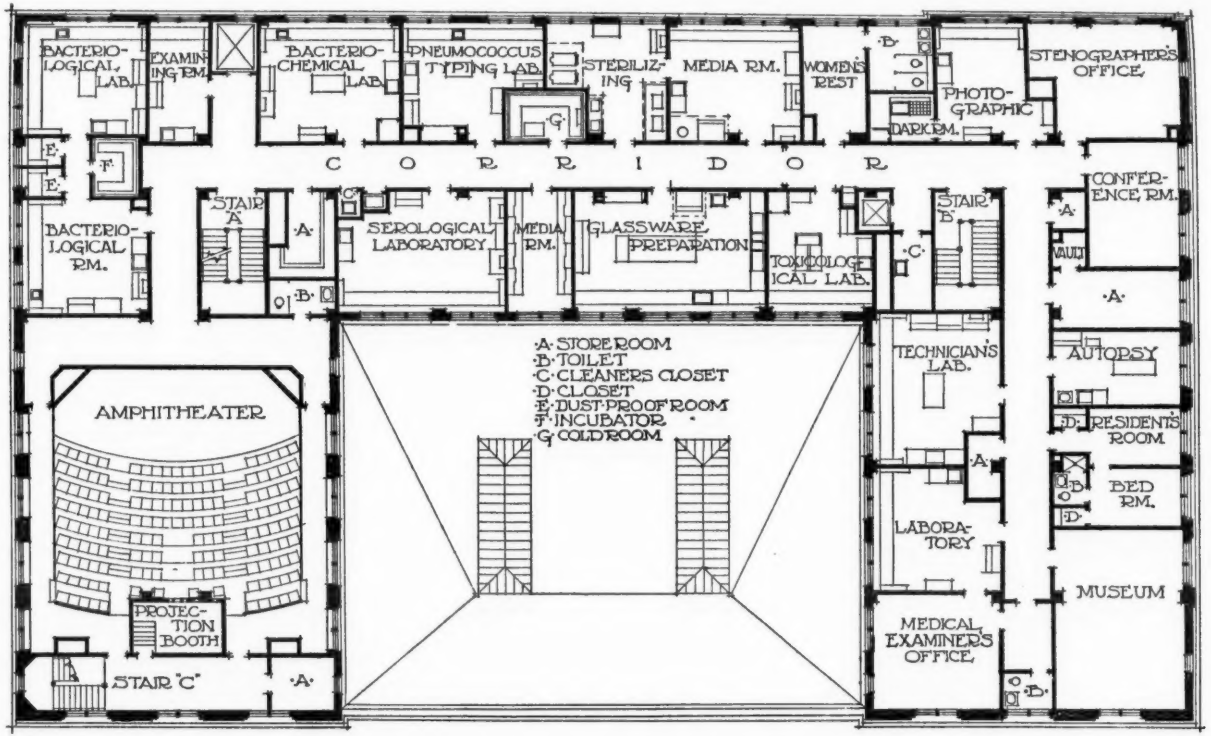
·MALLORY INSTITUTE OF PATHOLOGY · BOSTON CITY HOSPITAL · JAMES H. RITCHIE AND ASSOCIATES ARCHTS AND ENGR'S · BOSTON MASS.·

"transfer" rooms. These two rooms have air-tight doors and are ventilated by fans with automatic shutters, set into the upper sashes of the windows, air being drawn in through air filters. On the opposite side of the passageway is an electrically heated incubator room with suitable controls for maintaining an even temperature of 37.5° C. There is a small room which is used, chiefly, for examining patients and a larger one equipped with a chemical hood. A special room is provided for pneumococcus typing. It contains a built-in ventilated cabinet for holding mice jars. All the bacteriological rooms have small staining sinks built into the counters. The serological room contains the usual laboratory equipment, together with a generous supply of outlets to ensure adequate connections for various water baths.

Three rooms may be grouped as a sterilizing and glassware and media preparation unit. One room contains two autoclaves and hot-air ovens, an Arnold sterilizer and an inspissator. This connects directly with the media room, which, in addition to standard equipment, is provided with a 10 gallon steam-jacketed tilting kettle of Allegheny metal and a 6 burner gas range. Across the hall is a large room for the washing and preparation of glassware. There is a special sink with duplex washing and rinsing compartments, a small sink directly in front of one set of windows, a hopper, a large gas range and a steam-heated drying cabinet. The counter and table tops are covered with rubber to minimize breakage.

Set between the bacteriological group and this last group are two rooms for common use. The walls of one are lined with built-in cabinets for the storage of sterile glassware and media. The other is a cold room kept automatically at about 35° F. The latter contains a special freezing compartment cooled to about 20° F. and a standard counter with the usual service outlets.

The remainder of the second floor is devoted to the activities of the medical examiner. In addition to a private office and laboratory ample room is provided for stenographers, files and storage. There are a small chemical laboratory, a technicians' room, a photographic room with an adjoining darkroom, a private autopsy room, a museum and a women's restroom.



·SECOND FLOOR PLAN·

SCALE 5 10 15 20 FEET

Third Floor

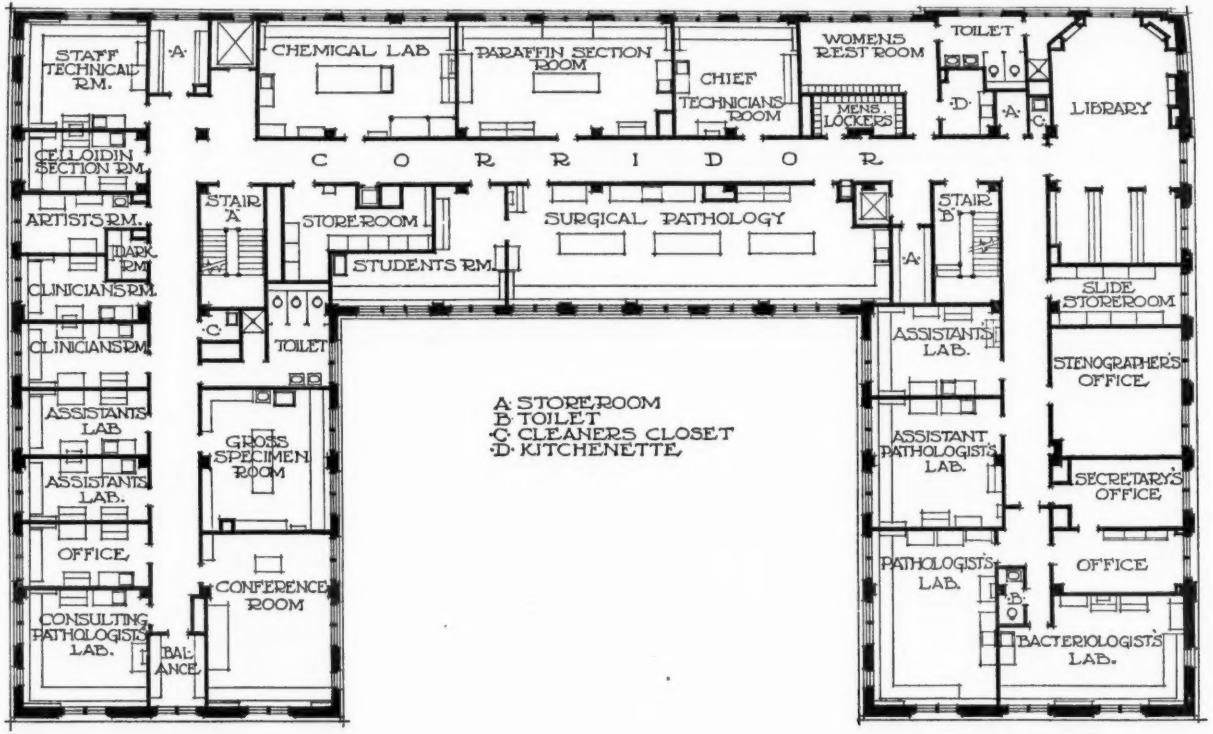
The entire third floor is assigned to the routine pathological work of the hospital.

In the extreme end of the north wing is a conference room with a seating capacity of about 30. This room is equipped with manually operated opaque shades and a screen for microprojection. At the front of the room are blackboards and the rear counter has an abundance of electrical outlets for microscopic demonstrations. Next to this room is a room for the storage of gross specimens. Pipe frame racks with alberene shelves are set against the walls for the agateware tanks. The counter under the windows and the large demonstration table in the middle of the room have alberene tops. The floor is of terrazzo and has a drain.

In addition to a balance room at the end of the corridor, the remainder of the wing is occupied by two large and seven small laboratories and a darkroom. The two larger rooms are used by the consulting pathologist and for a staff technical room, respectively. The smaller rooms are occupied by assistants and clinicians, with one devoted to the cutting of celloidin sections.

The north side of the main part of the building furnishes ideal natural light for microscopic work and the space is utilized for a large room for surgical pathology and a smaller one for special and graduate students, with generous counter space for 10 and 5 men, respectively. In the main room, at one end, are provisions for examining and preparing surgical specimens. The bottles of the celloidin series are kept on tables in the center of the room and there is a special sink with 20 washing outlets for use following Zenker fixation. Across the hall are a completely equipped chemical laboratory, a room for cutting paraffin sections, a room for the chief technician, a men's locker-room and a women's restroom with kitchenette attached.

The south wing is consigned to the staff and records. In connection with a secretary's room and office are the laboratories of the pathologist and bacteriologist. Two laboratories for the assistant pathologist and first assistant are provided. There is a library with a small stackroom, and next to the stenographers' and record room is one for slide storage cabinets.



·THIRD FLOOR PLAN·

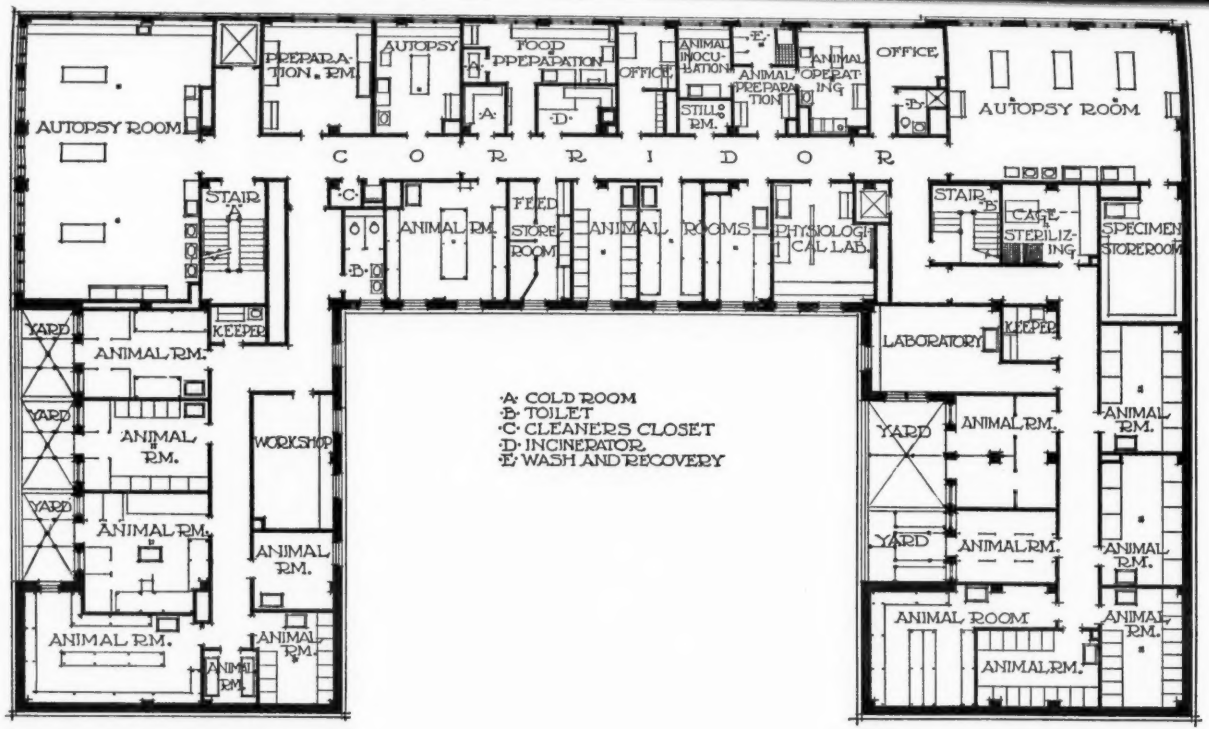
SCALE 0 5 10 15 20 FEET

Fourth Floor

Practically all of the north and south wings are used for stock animal rooms and outside pens. Each wing has a small room for the man in charge. The equipment of the individual rooms depends upon their purpose. Each is provided with a sink, sill cock and floor drain. Pressure steam is piped to each corridor and a hose of sufficient length to reach the far end of the farthest room is provided. Fixed sheet metal cages with mastic floors and set on concrete bases are furnished, in general, for rabbits, guinea pigs, dogs and cats. There are larger grilled pens for the dogs and grilled cages for monkeys and birds. Angle-iron racks suspended from the ceiling are furnished to hold individual cages for mice, rats, monkeys, birds and male rabbits. There are two inside and two outside pens for sheep. These wings also contain a workshop and a room for cage cleaning and sterilizing.

The main routine and medicolegal autopsy rooms are located at the north and south ends, respectively, of the main building. They have a higher ceiling (12 feet) than the other rooms and are lighted, chiefly, by large windows closely placed and running up to the ceiling. The floors are of a grayish green terrazzo with a greenish black border and base. The walls, above a 5 foot dado of light green matt glazed tile, topped by a narrow band of black, are white, as are the ceilings. Large white enamel sinks, lavatories and instrument sterilizers are fixed against the walls. The autopsy tables are built of stainless steel and the movable furniture, such as instrument cabinets, observation stands and tables, is finished in white enamel. Standard sets of service outlets are available on the walls near the autopsy tables and in the routine autopsy room a standard counter runs along one side of the room. The latter contains a staining sink and is provided with equipment for frozen sections. There is a small autopsy room near the main room, which is used for cases of contagious disease and for private autopsies. Between the two is a preparation room. All Zenker and gross fixation on autopsy material is completed in the latter. In connection with the medicolegal autopsy room are a small office and a room for storing gross specimens.

The central part of the main building houses a unit concerned, chiefly, with work on experimental animals. From an inner hall, used for animal preparation, open an inoculation room, an animal



FOURTH FLOOR PLAN

SCALE, 0 5 10 15 20 FEET

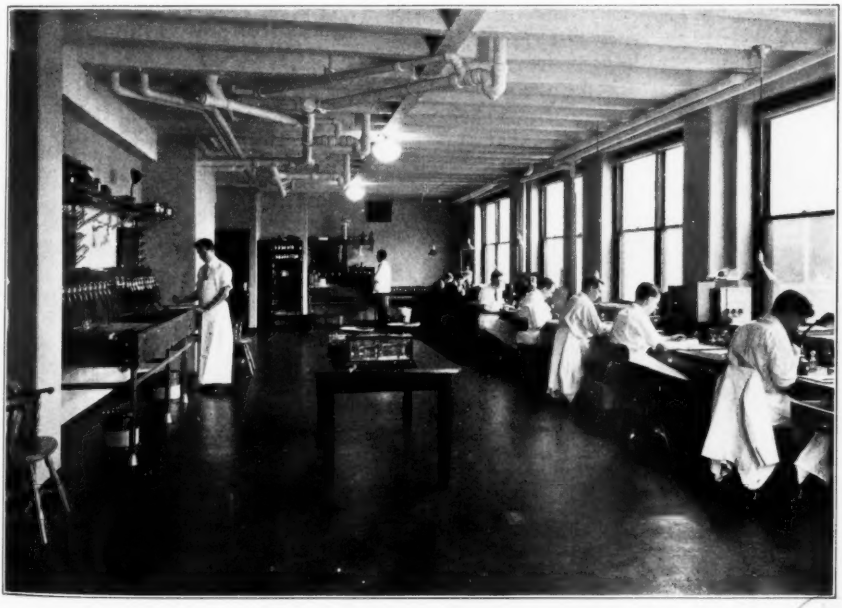
operating room completely equipped, and a small room for washing and postoperative recovery. A physiological laboratory contains, in addition to standard equipment, a floor trench with standard services, a wall panel for the mounting of heavy apparatus and A.C. electrical outlets for use with a portable roentgen-ray machine. There are four rooms for animals, varying in equipment according to their intended use, but conforming to the specifications of the rooms in the wings used for stock animals. There is a central office and locker room for the men in charge of the animals, a food preparation room with an adjoining cold room and a storage room for grain, hay and shavings. The incinerator room, still room, and a cold room for gross specimens are also included in this unit.

As a fitting recognition of the services that Dr. Frank Burr Mallory has rendered to the Boston City Hospital since the opening of the old Pathological Laboratory in 1895, the Trustees have named this building the Mallory Institute of Pathology. Its purpose is to conduct the routine pathological and bacteriological work of the Boston City Hospital and to investigate the cases referred to the medical examiner's office of Suffolk County, South, both in conjunction with undergraduate and graduate teaching.

Dr. Mallory, as consulting pathologist, continues on with the same activity and interest that he has always shown and his example and guidance serve as an ideal stimulus to the staff.

THE MAIN AUTOPSY ROOM

THE LIBRARY



THE ROOM FOR SURGICAL PATHOLOGY

CONTROLLED FORMATION OF COLLAGEN AND RETICULUM. A STUDY OF THE SOURCE OF INTERCELLULAR SUBSTANCE IN RECOVERY FROM EXPERIMENTAL SCORBUTUS *

S. Burt Wolbach, M.D.

(From the Department of Pathology, Harvard University Medical School, Boston, Mass.)

Previous studies [1] showed that in the state of complete or absolute scorbutus formation of intercellular materials cannot take place, and that following the administration of antiscorbutics easily demonstrable amounts of intercellular substance are formed within 24 hours. This rapid formation of intercellular substance occurs in regions where mesenchymal cells have accumulated in continuation of normal growth activities, in repair of spontaneous lesions in consequence of scorbutus and in the organization of blood clots after excision of tissue. These facts have made it possible to obtain and to recognize, in preparations from regions of growth and repair, intercellular substances deposited at will through the administration of antiscorbutics. Furthermore, we are enabled to locate exactly where collagen forms in relation to cells and fibrin and to follow the sequences in morphology of intercellular substances to the final or mature state.

Inasmuch as there are several conflicting beliefs concerning the source of collagen and its relation to cells this communication is restricted to observations that are of value as premises for the solution of this problem.

Although a large amount of material from many guinea pigs was on hand, covering all stages in the repair of spontaneous lesions in scorbutus, a new series of guinea pigs was prepared in order to take advantage of the isolation of connective tissue cells which occurs in the avascular organization of blood clots in animals in absolute scorbutus. Absolute scorbutus we define as the stage in experimental scorbutus in which formation of intercellular materials either in growth or repair has ceased. Proliferative reparative cellular responses, epithelial and mesenchymal, are active. It is always accompanied by marked osteoporosis.

* Received for publication September 10, 1933.

The diet was that previously employed by Wolbach and Howe.[1] Guinea pigs of from 315 to 400 gm. initial weight were used. On the 23rd day of the vitamin C free diet a large piece was removed from the extensor thigh muscles of each animal, under aseptic precautions, and the skin incision sutured. The ensuing blood clot was allowed to undergo organization for periods of from 5 to 8 days before administration of orange juice. The duration of treatment with orange juice, before sacrificing the animal, varied from 24 to 96 hours. Control guinea pigs, *i. e.*, those that received no orange juice following the removal of muscle, were killed at the end of 7 to 10 days. From each animal, immediately after death, the experimental wound was excised, with the minimum amounts of surrounding normal tissues, so as to ensure the best possible fixation. The costochondral junctions were also saved and sectioned. Zenker's fixative was used for both types of material. Twenty-four hours fixation was sufficient to decalcify the osteoporotic ribs. All tissues were embedded in paraffin. The stains employed were a modified Giemsa stain [2] for general study, Mallory's phosphomolybdic acid anilin blue- acid fuchsin- orange G connective tissue stain,[3] Mallory's phosphotungstic acid hematoxylin,[3] and Foot's modification of the Bielschowsky-Maresch silver impregnation method [4] followed by Van Gieson's acid fuchsin-picric acid stain, or by Mallory's connective tissue stain.

The repair of the blood clot in absolute scorbutus begins promptly by the migration of fibroblasts from adjacent tissues into the clot, and the continued division of these cells. The fibroblasts are unaccompanied by collagen formation and they retain shapes resembling those of embryonic connective tissue cells, or fibroblasts in tissue plasma cultures (Fig. 1). Capillaries do not penetrate the clot for any considerable distance and are apparently unable to form, although closed columns of endothelial cells accompanied by fibroblasts do form. Islands of erythroblastic cells appear in the peripheral zone of the clot and in the adjacent tissue about capillaries, and apparently are derived from vascular endothelial cells whose initial reparative response to the experimental wound is multiplication. The fibroblasts apply themselves closely to fibrin when the latter is present. They probably follow fibrin strands in their movements (Fig. 2). The behavior of the cells invading the blood clot and the

microscopic picture they produce are similar to those arising in tissue plasma cultures. Fibroglia fibrils are present and stain sharply in absolute scorbutus (Figs. 2 and 9). No collagen or reticulum can be found in any attempt at repair during absolute scorbutus. As shown in Figure 2, the fibroblasts in such granulation tissue are never in close contact and very frequently they are separated by vacuoles outlined by very delicate acidophilic material which is often granular. This appearance of edema is also present in the *Gerüstmark* zone of the costochondral junction and in clusters of fibroblast-like cells on the internal surface of the resorbing cortical bone of ribs in scorbutus. In preparations stained with Mallory's connective tissue stain there is often a faint bluish coloration to the cytoplasm of fibroblasts in organizing blood clots in scorbutus and to the peripheries of the vacuoles between cells. The appearances indicate that in absolute scorbutus fibroblasts are responsible for the formation of an extracellular liquid. The fibroblasts (Fig. 2) themselves are vacuolated usually at their extremities, particularly at points of divergence of fibroglia fibrils, and a frequent picture is that of fibroblasts with spongy cytoplasm whose boundaries are delineated only by the aid of the fibroglia fibrils upon their surfaces. These appearances suggest that the contents of these vacuoles are discharged and accumulate to some degree between the cells. It is to be remembered that these observations apply to cells in regions remote from blood vessels and lymphatics, so that transudation as a source of edema is probably excluded. There can be little ground for denying that the extracellular liquid has its source in the cytoplasmic vacuoles. The vacuoles may be evidence of degeneration or the consequence of excessive secretion of an abnormal cell product; this study supports the latter interpretation.

The presence of collagen following administration of orange juice (10 to 15 cc. daily) was studied at 24, 48, 72 and 96 hour periods. For the sake of brevity the term collagen is used to include the material first deposited around fibroblasts in repair, including the intercellular material first laid down in the formation of bone. It includes so-called reticulum (argyrophile fibrils), as determined by silver impregnation methods, as well as the material having the normal staining properties of collagen with the methods employed in this study. Collagen and reticulum were found around cells, often completely isolated, far from blood vessels and preëxisting normal

tissues. The deposit of collagen appeared later and less abundantly about those cells that had penetrated farthest into the blood clot, so that deposits of different ages could be identified in single preparations. The collagen was found only in immediate contact with cells. The distribution of the first deposit is determined by the shape of the cell and influenced by the proximity of other fibroblasts. The collagen about isolated cells, far in the blood clot, surrounded the individual cell and did not radiate or extend into the clot away from the cell. The same applies to fibrils impregnated with silver by Foot's modification of the Bielschowsky-Maresch method. This restricted localization of the collagen was most strikingly evident about rounded, non-processed fibroblasts that had come to rest deep in the clot (Fig. 4).

Elongated cells with processes showed collagen distributed in all the directions of the cell processes and as far as the fibroglia fibrils extended. Elongated cells in contact with fibrin showed collagen on all sides of the cell (Fig. 3), and the fibrin remained unaltered during the early periods of collagen formation.

No appearance remotely suggestive of the transformation of fibrin into collagen was found, or that fibrin contributed in any manner to collagen formation. One observation which might possibly be construed as evidence for a participation of fibrin in collagen formation is that excavation of large fibrin masses by fibroblasts is more conspicuous after collagen formation has been induced (Figs. 5 and 6). This, however, may be explained by the restoration of enzymatic functions to the cell. There is evidence that the rôle of the fibroblast and osteoblast in collagen formation is a reversible one, for the collagen deposited in the early repair of bone in recovery from scorbutus may later be resorbed about these same cells. Whether or not the products of fibrin digestion assist in the formation of collagen, beyond providing sustenance to the cells, cannot be decided by these experiments, although it may be positively asserted that fibrin is not essential to collagen formation and that direct transformation into collagen (or reticulum) does not occur.

The first material having the staining properties of collagen which appeared around cells was homogeneous, resembling lightly stained amyloid and therefore presumably not of great density. It was best observed where fibroblasts were grouped closely together and between fibroblasts and fibrin strands (Figs. 3 and 5). This homo-

geneous state was of short duration, though best seen on the second day when collagen deposition was rapid; no extensive zones of it were found. It was intimately applied to the cytoplasm of the cell body or to fibroglia fibril extensions beyond apparent limits of the cell. Its site of formation in relation to the cell body was always external to fibroglia fibrils. It was present about cells when no reticulum or argyrophile fibrils could be demonstrated.

Next in sequence was the appearance of delicate fibrils coincidently with the appearance of reticulum, or argyrophile fibrils (Figs. 5 and 6). With isolated, rounded or ovoid cells the direction of the fibrillary collagen, as well as that of argyrophile fibrils, was concentric to the cell body; if the cells were processed the fibrils paralleled the cell processes and fibroglia fibrils. In cell clusters the fibrils coursed irregularly between cells and it was impossible to associate any group of fibrils with a definite cell (Fig. 7).

Early in recovery from scorbutus, up to 72 hours (Fig. 6), the reticulum method gave the appearance of there being exceedingly delicate black fibrils embedded in a homogeneous collagen matrix, producing the effect of broad bands of black reticulum. This microscopic sequence may be explained as fibril formation in a homogeneous material. More mature collagen in later periods of recovery does not impregnate with silver. The first detectable argyrophile material appears in the form of black granules at the limit of microscopic resolution, arranged in rows, always somewhat tortuous, never straight. Identical sequences were found in the *Gerüstmark* zone of the ribs and in the resumption of osteoid deposit in the endochondral formation of bone (Fig. 8) and upon the inner ("endosteal") and outer (periosteal) deposition of osteoid.

The conclusion is unavoidable that the earliest formed collagen fibrils are identical with reticulum and that the property of impregnating with silver is due to delicacy of the fibrils, a conclusion reached by Mallory and Parker [5] from a study of human tissues and tumors, by Alfejew [6] from the study of mammalian embryos, and by Maximow [7, 8] from the study of rabbit tissue plasma cultures.

In the resumption of bone formation in recovery from scorbutus sequences identical to those described above take place, and the first deposit of intercellular material cannot be differentiated from that which forms in organizing blood clots and in other regions not destined to ossification.

The study of bone resorption in regions of most marked osteoporosis in absolute scorbutus offers convincing evidence that some liberated bone cells survive and increase in size and acquire appearances usual to fibroblasts. Fibroglia fibrils can be seen in enlarged bone canaliculi and to extend into the enlarged marrow cavity. Traces of matrix extending inward from the cortical bone lie parallel to fibroglia fibrils. The lacunae in which bone cells lie are enlarged (Fig. 9). These facts are mentioned because they are some of the observations that indicate resorption of bone matrix and its collagen constituent is dependent upon the activity of those cells that under other conditions are concerned in its formation. They also indicate that the fibroglia fibrils are conductors of the agents concerned in resorption as well as in the deposition of matrices, and that fibroglia fibrils may have a cytoplasmic vesture. In recovery from scorbutus (Fig. 10) prompt formation of intercellular substance at first indistinguishable from collagen by reticulum staining methods and the usual connective tissue stains took place. As in the case of collagen formed in the organization of blood clots the deposit in bone formation was at first homogeneous, then fibrillary, and its distribution determined by cells and their processes. Studies which will not be presented here support the belief in the existence of a homogeneous material enveloping the collagen fibers in the completed bone matrix.

Summary and Discussion

The organization of blood clots during the state of absolute scorbutus in guinea pigs is a process in which fibroblasts become widely separated from their sources and from blood vessels, capillaries and lymphatics. Fibroblasts may also be found separated from one another, migrating and dividing as in tissue plasma cultures, following strands of fibrin and penetrating masses of blood corpuscles in regions where stainable threads of fibrin are absent. Collagen does not appear either as reticulum or in more easily demonstrable form until recovery is induced by the administration of an antiscorbutic. Under the conditions of these experiments the exact situation of reticulum and collagen formation has been determined.

Collagen appears first as a homogeneous material in which argyrophile or reticulum fibrils promptly appear. Coincidently with the appearance of the argyrophile fibrils the stains in common usage for

demonstrating collagen show the presence of collagen fibril bundles. The distribution of the collagen is dependent upon the form of the cell and with isolated cells is confined to zones immediately adjacent to the cell body and its processes, including the entire length of fibroglia fibrils.

The course or direction of the collagen and argyrophile fibrils is parallel to surfaces of the fibroblast and its processes. Because of this arrangement parallel to the cell and its processes, and never radiating, fibril formation must be influenced by factors not present in the formation of fibrin strands from plasma, and speculation is suggested concerning a molecular alignment previous to the development of fibrils. Further speculation suggests that the pattern of collagen fibrils formed rapidly in groups of cells which are without processes is influenced by many cells as by a resultant of forces acting in the homogeneous or amorphous stage of collagen formation. Where the processes and fibroglia fibrils of fibroblasts interweave the appearances are that each cell is accompanied by collagen fibrils in parallel arrangement to its ramifications.

Careful study of regions containing fibrin in experimentally produced clots and in regions of spontaneous injury, particularly at the line of infraction in ribs, yielded no suggestion of transformation of fibrin into collagen. The presence of fibrin does not modify the arrangement of collagen about the fibroblasts, which is conclusive evidence that fibrin does not directly contribute to the formation of collagen.

Baitsell,[9–12] both in tissue plasma cultures and in the formation of tuberculous tissue in the guinea pig testis, has presented evidence for the transformation of fibrin and exudate into collagen. His material was so different from that of the present study that it is impossible to exercise critical judgment in comparison of conclusions. It may be said that the conditions in tuberculous testes of guinea pigs are exceedingly complex, as compared to the recovery phenomena in organization of blood clots in scorbutus.

Nageotte,[13] in many types of experiments, concludes that fibrin is transformed into collagen, but also states that other albuminoid substances, including the protoplasm of dead cells, may be precursors of collagen.

In embryos a common and well substantiated origin of collagen is "a transparent, gelatinous, cell-free, ground substance which, in

general, pervades the embryonic body from very early stages of development."[15] The recent papers of Baitsell,[14, 15] Harrison,[16] and Alfejew [6] all express the opinion that fibrils form in this ground substance independently of the immediate proximity of cells. Maximow and Bloom [8] describe spiral argyrophile fibrils formed in tissue cultures remote from cells. This observation seems to have been made by Bloom, as it does not appear in Maximow's publication [7] based upon the same material.

An early conception of the origin of collagen fibrils was the direct modification of the cytoplasm of the surface of the fibroblast and its processes, held by Hansen,[17] and Mall,[18] and other noted investigators. The most substantial recent support of this view is that of M. R. Lewis [19] from tissue culture studies. According to Lewis the fibrils appeared in cultures after 24 hours growth, "as slightly more refractive lines within the cytoplasm of the individual cell" and later became gathered into bundles outside of the cell to form slender fibers. Mitochondria were described as being occasionally carried by fibrils to points outside of the cell, though not concerned in fibril formation, as has been held by Meves,[20] and others. The conclusions of this impressive paper cannot be reconciled with those of the present study. A further divergence is Lewis' conclusion that the vacuoles which form in fibroblasts are not concerned in fibril formation, as first suggested by Péterfi [21] in the study of argyrophile fibrils in epithelial cells of the amnion of fowl embryos.

Whether or not the vacuoles that form in fibroblasts in the scorbutic guinea pig have a counterpart in fibroblasts in granulation tissue under normal conditions has not been determined. Their presence in scorbutus may be regarded as pathological, but also as due to an exaggeration of a normal process, and in all probability as the source of the extracellular liquid which is so conspicuous in certain regions in experimental scorbutus and which may represent a liquid precursor of collagen, as suggested by the rapid appearance and large volume of intercellular material in the recovery from scorbutus.[1]

In the above discussion reference has been made only to papers with a bearing upon observations made in the present study. Excellent reviews covering the literature of collagen formation are to be found in Lewis' paper [19] and by Maximow in Möllendorff's Handbuch.[22] Relevant to the subject, as concerning intercellular substances, are the recent articles with exhaustive reviews of the

literature on cartilage by Schaffer,[23] and on bone by Weidenreich,[24] also in Möllendorff's Handbuch.

Consideration of the three theories held in explanation of collagen fibril formation — (1) the intracellular by transformation or metaplasia of the surface cytoplasm (ectoplasm) with or without the participation of mitochondria; (2) the origin in an extracellular amorphous ground substance secreted by cells, and (3) the transformation by enzymes of extracellular material not produced by fibroblasts, such as fibrin or other forms of exudate and albuminoid materials from dead cells — shows compatibility of only the second with the results of the present study. In embryos large amounts of amorphous precollagen are formed and fibril formation apparently takes place in regions remote from cells. In Maximow's tissue cultures the first evidence of intercellular materials was the formation of argyrophile fibrils, apparently not necessarily in close contact with cells and extending into the medium without relation to cell processes.

In recovery from scorbutus the collagen deposit is restricted to the immediate vicinity of fibroblasts. It is at first amorphous. Argyrophile fibrils appear first in the earliest deposited material; this can be best seen in the resumption of bone matrix formation in endochondral bone formation (Fig. 8). About fibroblasts the collagen distribution is definitely related to the cell body and its processes, including the fibroglia fibrils. The study of resorption of bone and the shapes assumed by released bone cells indicates that the fibrils of the latter are identical with fibroglia fibrils and that they in some manner are active in the resorption of bone matrix. The distribution and directions of collagen fibrils in recovery from scorbutus are also influenced by fibroglia fibrils. In addition to possible mechanical functions fibroglia fibrils are conductors of agents presumably having reversible enzyme activities.

The writer's evaluation of the conclusions drawn from this study is influenced by unpublished studies of sequences in resumption of growth of bone in recovery from rickets, scorbutus and vitamin A deficiency. In each instance the reparative processes were identical with the normal sequences that the deficiency had suspended, but they proceeded, in their early stages, at a rate far beyond that of normal growth. This is probably true of recovery phenomena from all vitamin deficiencies and seems definitely so in the resumption of collagen formation by fibroblasts in the present study.

Conclusions

1. Fibrin and other preformed materials do not contribute to collagen formation in repair by organization.

2. Collagen and reticulum represent physical differences of the same material.

3. Collagen is the product of secretory activity of fibroblasts, and its alignment and distribution are determined by the shape of the cell and its processes, including fibroglia fibrils.

References

1. Wolbach, S. B., and Howe, P. R. Intercellular substances in experimental scorbutus. *Arch. Path. & Lab. Med.*, 1926, **1**, 1–24.

2. Wolbach, S. B. Studies on Rocky Mountain spotted fever. *J. Med. Res.*, 1919–20, **41**, 1–197.

3. Mallory, F. B., and Wright, J. H. Pathological Technique. W. B. Saunders Company, Philadelphia and London, 1924, Ed. 8.

4. Foot, N. C. A technic for demonstrating reticulum fibers in Zenker-fixed paraffin sections. *J. Lab. & Clin. Med.*, 1923–24, **9**, 777–781.

5. Mallory, F. B., and Parker, Frederic, Jr. Reticulum. *Am. J. Path.*, 1927, **3**, 515–525.

6. Alfejew, S. Über die embryonale Histogenese der kollagenen und reticulären Fasern des Bindegewebes bei Saügetieren. *Ztschr. f. Zellforsch. u. mikr. Anat.*, 1925–26, **3**, 149–168.

7. Maximow, A. Development of argyrophile and collagenous fibers in tissue cultures. *Proc. Soc. Exper. Biol. & Med.*, 1927–28, **25**, 439–442.

8. Maximow, A., and Bloom, W. Über die Entwicklung argyrophiler und kollagener Fasern in Kulturen von erwachsenem Saügetiergewebe. *Ztschr. f. mikr.-anat. Forsch.*, 1929, **17**, 625–658.

9. Baitsell, G. A. The origin and structure of a fibrous tissue which appears in living cultures of adult frog tissues. *J. Exper. Med.*, 1915, **21**, 455–479.

10. Baitsell, G. A. The origin and structure of a fibrous tissue formed in wound healing. *J. Exper. Med.*, 1916, **23**, 739–756.

11. Baitsell, G. A. The transformation of the plasma clot. *Am. J. Physiol.*, 1916, **42**, 608–609.

12. Baitsell, G. A., and Mason, K. E. The origin of the fibrous tissue arising in the testis of the guinea-pig following experimental tuberculosis. *Am. Rev. Tuberc.*, 1930, **21**, 593–626.

13. Nageotte, J. L'organisation de la matière dans ses rapports avec la vie. Félix Alcan, Paris, 1922, 47.

14. Baitsell, G. A. On the origin of the connective-tissue ground-substance in the chick embryo. *Quart. J. Micr. Sc.*, 1924–25, **69**, 571–589.

15. Baitsell, G. A. A study of the development of connective tissue in the amphibia. *Am. J. Anat.*, 1920–21, **28**, 447–467.

16. Harrison, R. G. The development of the balancer in amblystoma, studied by the method of transplantation and in relation to the connective-tissue problem. *J. Exper. Zool.*, 1924–25, **41**, 349–427.

17. Hansen, F. Über die Genese einiger Bindegewebsgrundsubstanzen. *Anat. Anz.*, 1899, **16**, 417–438.

18. Mall, F. On the development of the connective tissues from the connective-tissue syncytium. *Am. J. Anat.*, 1901–02, **1**, 329–365.

19. Lewis, M. R. Development of connective-tissue fibers in tissue cultures of chick embryos. *Contrib. Embryol.*, 1917, **6**, 45–60.

20. Meves, F. Über strukturen in den Zellen des embryonalen Stützgewebes sowie über die Entstehung der Bindegewebsfibrillen, insbesondere derjenigen der Sehne. *Arch. f. mikr. Anat.*, 1910, **75**, 149–208.

21. Péterfi, T. Beiträge zur Histologie des Amnions und zur Enstehung der fibrillären Strukturen. *Anat. Anz.*, 1913–14, **45**, 161–173.

22. Maximow, A. Bindegewebe und blutbildende Gewebe. Handbuch der mikroskopischen Anatomie des Menschen, von Möllendorff, W. J. Springer, Berlin, 1927, **2**, Pt. 1, 232–583.

23. Schaffer, J. Die Stützgewebe. Handbuch der mikroskopischen Anatomie des Menschen, von Möllendorff, W. J. Springer, Berlin, 1930, **2**, Pt. 2, 1–390.

24. Weidenreich, F. Das Knochengewebe. Handbuch der mikroskopischen Anatomie des Menschen, von Möllendorff, W. J. Springer, Berlin, 1930, **2**, Pt. 2, 391–520.

DESCRIPTION OF PLATES

PLATE 112

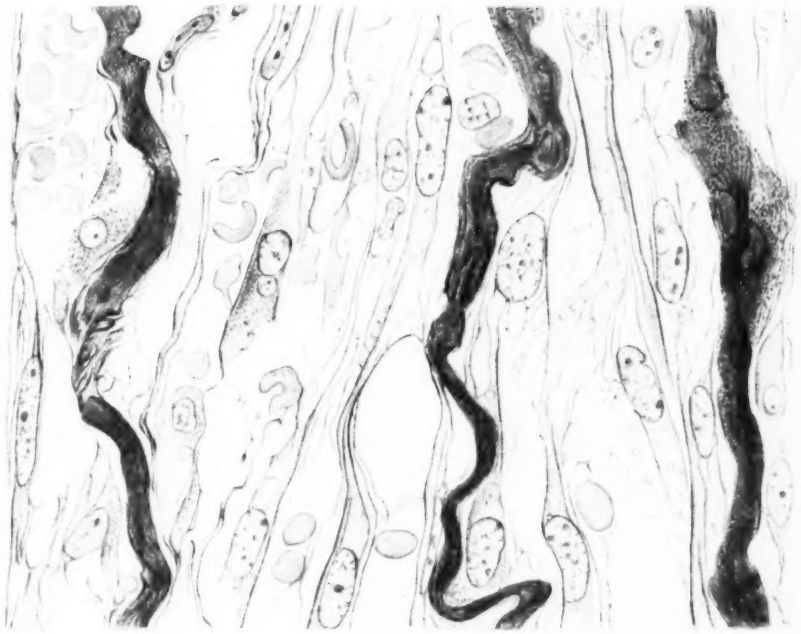
FIG. 1. Repair by avascular organization in absolute scorbutus. A strand of fibrin crosses the upper right-hand corner. Most of the cells in this field have probably emigrated from connective tissue surrounding the nerve shown in the lower border. Most of the fibroblasts are separated from one another. Five mitotic figures are in this field. Guinea pig operated upon on 23rd day of vitamin C free diet; killed on 30th day of deficiency. Initial weight 315 gm., maximum weight 360 gm., and final weight 204 gm. Modified Giemsa stain. × 125.

1

PLATE 113

FIG. 2. Fibroblasts in relation to fibrin strands in organization in absolute scorbutus. Fibroglia fibrils are prominent. Note vacuoles in cytoplasm of the fibroblasts and extracellular vacuoles described in the text. No stainable collagen, including argyrophile fibrils, present. Guinea pig operated upon on 23rd day of deficient diet; killed on 33rd day of the deficiency. Initial weight 400 gm., maximum weight 446 gm., final weight 320 gm. Mallory's connective tissue stain. × 1000.

FIG. 3. To illustrate the collagen formed in organization tissue during a 40 hour period of recovery from absolute scorbutus. The region illustrated is deep in the blood clot. Note homogeneous appearance of the collagen surrounding cells in contact with fibrin in left third of the field; fibrillation of collagen is apparent elsewhere. Other preparations stained by Foot's method showed argyrophile fibrils in small numbers and corresponding to the fibrils illustrated. Guinea pig operated upon on 23rd day of deficient diet; orange juice administered on afternoon of 28th day; killed on morning of 30th day of the experiment. Initial weight 357 gm., maximum weight 395 gm., final weight 301 gm. Mallory's connective tissue stain. × 1000.



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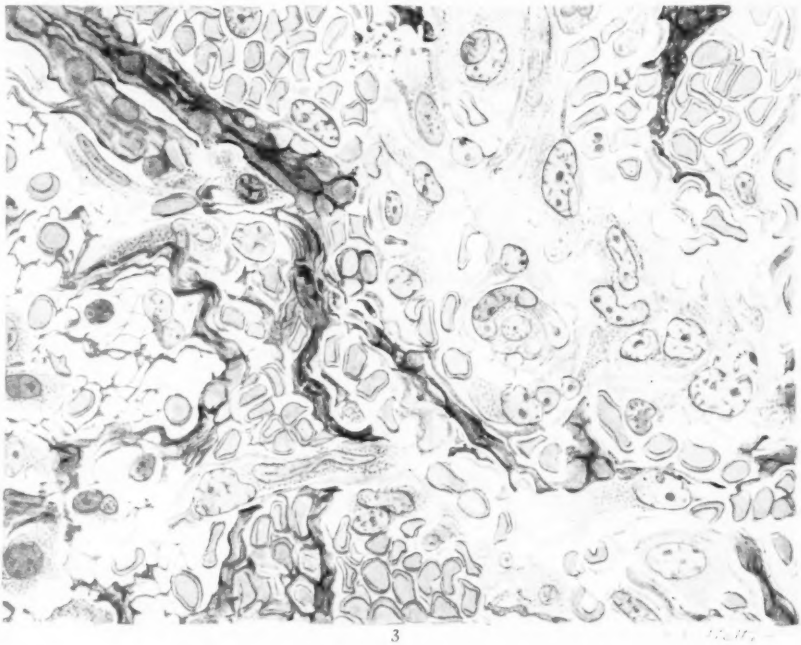


3

Wolbach Controlled Formation of Collagen and Reticulum

PLATE 114

FIG. 4. To show collagen in relation to an isolated cell deep within the blood clot. Recovery period of 72 hours. Guinea pig operated upon on 23rd day of deficient diet, orange juice first given on 31st day of experiment. Initial weight 395 gm., maximum weight 418 gm., final weight 288 gm. Mallory's connective tissue stain. × 1000.

FIG. 5. Recovery period of 72 hours; same animal used for Fig. 4. A field from a group of cells deep within the clot, illustrating collagen deposited by cells that have penetrated fibrin strands. Mallory's connective tissue stain. × 1000.

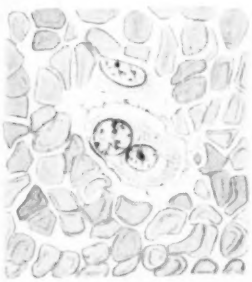
FIG. 6. Recovery period of 72 hours. This field corresponds to that of Fig. 5 and is from the same block at a slightly different level. The argyrophile fibrils have formed and their distribution in relation to the cells is the same as that of the collagen. Foot's modification of Bielschowsky-Maresch silver impregnation method, followed by Mallory's connective tissue stain. × 1000.

FIG. 7. To illustrate collagen formed by a cluster of cells during a 96 hour repair period. Guinea pig operated upon on 23rd day of deficient diet; orange juice administered from 31st day to 35th day inclusive; killed on 36th day of experiment. Mallory's connective tissue stain. × 1000.

FIG. 8. To illustrate resumption of endochondral bone formation. Rib, recovery period of 40 hours duration. Preparation from the same guinea pig used for Figs. 3 and 10. To illustrate newly formed argyrophile fibrils in presence of homogeneous collagen representing the first stage in formation of the intercellular substance of bone. Argyrophile fibrils have formed in the amorphous collagen first deposited by the immature osteoblasts. Staining technique same as that of Fig. 6. × 430.

FIG. 9. "Endosteal" surface of rib of guinea pig in absolute scorbutus. To illustrate details of bone resorption described in the text. Note the appearances of cells indistinguishable from fibroblasts which in recovery from scorbutus become osteoblasts. The yellow mottling of the bone is characteristic of advanced scorbutus, when stained by Mallory's method. Note the continuity of fibroglia fibrils emerging from the cortex with the fibroblasts in the cavity of the bone. Mallory's connective tissue stain. × 1000.

FIG. 10. To illustrate early "endosteal" bone formation during a 40 hour recovery period and relation of the collagen to cell shapes and fibroglia fibrils. Rib, from guinea pig used for Figs. 3 and 8. Mallory's connective tissue stain. × 1000.



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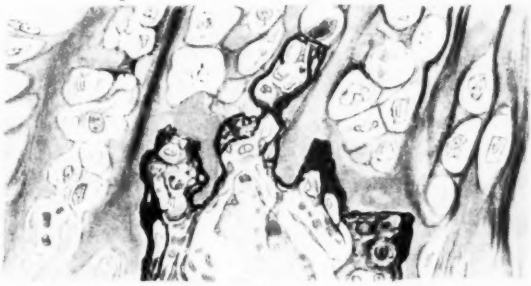

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Wolbach

Controlled Formation of Collagen and Reticulum

THE CANCER CELLS OF SEROUS EFFUSIONS *

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Free tumor cells appear commonly in the fluid accumulations consequent upon neoplastic disease of the serous cavities. Their recognition is of practical importance through its value in facilitating clinical diagnosis in cases otherwise obscure. These cells are also of much interest from the standpoint of general cytology and oncology. Known for many years, they have been studied for the most part only in smear preparations or in the wet state. The resulting uncertainties have bred a laboratory tradition highly suspicious of any attempt at their positive identification. They may, however, be seen clearly in preparations secured by centrifugation of fresh aspirated fluid, followed by Zenker fixation, paraffin embedding and staining of the centrifugate. This simple method was discovered a number of years ago. It appears to have been developed independently by several workers [1] and the general procedure was described by Mandlebaum [2] as long ago as 1917.

The present observations are based upon the study of some fifty cases of implanted or metastatic pleural or peritoneal carcinoma encountered in the course of routine diagnostic work during the past ten years. The preparations have been stained by hematoxylin and eosin or eosin-methylene blue, supplemented at times by iron hematoxylin or Mallory's phosphotungstic acid hematoxylin and aniline blue connective tissue stains. The material has been prepared and fixed as soon as possible after its withdrawal from the body, but cell preservation has been surprisingly good even in some fluids fixed 24, or even 48 hours later. Diagnostic findings have been obtained occasionally on small amounts of the aspirated fluid. In some cases as little as 10 to 15 cc. have provided a centrifugate mass permitting definite conclusions.

Several types of cell arrangement are present in the different fluids. Easiest of recognition as products of neoplastic proliferation

* Received for publication June 9, 1933.

are microscopic fragments of actual tumor tissue. Particles with epithelium and stroma have been seen in three cases, two of them presenting obvious papillary structure. In two other cases there are disintegrating remnants of colloid carcinoma. These are the only instances in which stroma elements are found in association with epithelium. In a peritoneal effusion due to gastric carcinoma there are a few groups of epithelial cells unaccompanied by stroma but showing plainly a suggestion of alveolar arrangement, while traces of mucus cling to the walls of the suggested lumens. In three fluids from the pleural cavity, one following bronchiogenic carcinoma, and two consequent upon metastasis from mammary carcinoma, there are large clumps of cells which, in the sectioned preparation, have a pavement-like arrangement of large units, the picture suggesting squamous epithelium. Cytoplasmic and nuclear structure are sufficient to establish the conclusion that these have nothing to do with the benign "endothelial plaques" of the early French writers. In each case there is the confirmatory evidence of clear-cut mitotic figures.

Mitosis may be infrequent or very prominent (Fig. 4). It is found in isolated cells as well as in individual elements of cell groups. Its occurrence, together with other evidences of good cell preservation and the peculiar organoid arrangements sometimes encountered, is believed to indicate that the neoplastic cells are proliferating within the serous fluid and not merely floating in it as elements desquamated from the surface of mural tumor masses. It may be assumed that these free cells are to some extent removed from the regulating influence of such factors of tissue balance as may apply to growth within the solid tissues. On the other hand, they differ from the cells of artificial tissue cultures in possessing nutritional and environmental conditions more nearly approaching the normal than is possible for cells growing *in vitro*. They might, therefore, be expected to provide an opportunity for the study of tumor cells from an angle somewhat different from the usual.

In several of the fluids there are formations that show very clearly their descent from glandular epithelium. Three of the six instances encountered were ascitic effusions following peritoneal implantation of cystic adenocarcinoma of the ovary and a fourth was probably of ovarian origin, although laparotomy failed to reveal the original tumor site. Another peritoneal effusion followed metastasizing

adenocarcinoma of the fundus uteri. One well defined example occurred in a pleural effusion, but unfortunately the site of origin of the tumor could not be checked by autopsy. These fluids contain aggregations of epithelial cells free of stroma but exhibiting distinct adenomatous arrangement. Some of the groups may appear as clumps of columnar elements with disintegrative changes in the centrally located cells. Many are hollow vesicles, the cavity empty or containing a few loose cells and occasionally colloid droplets or a faint network or linear pattern of fine lines suggesting the presence of mucinous residue. In one specimen the prevailing arrangement is that of clear-cut epithelial circlets with relatively large clear central spaces. Serial sections prove them to be hollow spheres or vesicles with a single layered wall that may, however, contain occasional outward-bulging clumps of irregularly arranged cells (Fig. 1). The cells of the vesicle wall may be flattened, cuboidal or polygonal and occasionally distinctly columnar. They may be well preserved or vacuolated. Wide variations in morphology occur even within the individual vesicle. Along with the vesicle type of formation there appear in one specimen groups of columnar cells in acinar or tubular arrangement of strikingly organoid appearance. The organoid structure is particularly marked in one specimen where many of the groups contain one to several mitotic figures. The vesicle arrangement was seen in one case in the wet unfixed material. It was noted long ago by Ehrlich and by Beneke,[3] but the wet preparation or smear gives only a very imperfect idea of the structural details.

In over half the cases of the series the tumor cells occur singly or in small groups of only a few units (Fig. 2). Here, also, their neoplastic character may be clearly evident but occasionally becomes difficult of demonstration. The difficulty lies in the long recognized problem of their differentiation from the large cells seen in fluids of neoplastic as well as inflammatory origin and usually accepted as mesothelial. The identity of these cells needs further working out by modern methods. As they are observed in some cases in close association with tumor cells and equalling, or even exceeding them in number, the following question arises. Are they stimulated to overproduction by the tumor growth, or is the relation reversed and do they serve like Carrel's "nurse cells" of tissue cultures [4] to provide food or activating factors for the neoplastic cells? As a matter of practical differentiation, it is only rarely that careful search of the

transudate resulting from neoplastic growth will fail to reveal single cells, or cell aggregations that betray their tumor origin, even when these cells are far outnumbered by leukocytes, or by elements of doubtful type. Divergence from the normal takes the direction of irregularity in size, and particularly of increased size of the cell, enlargement and hyperchromatism of the nucleus, and increased prominence of the nucleolus. Mitosis is, of course, highly significant. It is doubtful whether this occurs in other than neoplastic cells with sufficient frequency to complicate the problem, despite such conclusions as those of Karp,[5] who found it often in cells accepted as mesothelial in smear preparations. Fat droplet accumulations and hydropic or other vacuolation and disintegrative changes indicative of metabolic disturbance may be common in tumor cells as well as in the other types present. Coarse vacuolation often results in the formation of signet ring figures. The signet forms of the neoplastic cells may consist of two or more cells and, as believed by Karp and previous observers, tend to be larger than those of mesothelial origin.

A common finding, which remains as difficult of interpretation as it was in the early days of pathological cytology, is the incorporation within the cytoplasm of a tumor cell of one or more other cells, themselves definitely neoplastic. These are the mother cells, regarded by Virchow as producing daughter cells by endogenous proliferation. The included cells have been considered, on the other hand, merely as invaginations. As seen in the present preparations, they seem to be definitely within and not merely pressed against the larger cytoplasmic mass. Within the solid tissues such forms might readily be interpreted as the result of mutual pressure, but such origin is not likely here. By contrast, leukocytic invasion of the cytoplasm is uncommon. The included cells may be closely enveloped or may lie in a vacuolar space. While often well preserved, or even in mitosis, they are apt to be small, with more or less evident cytoplasmic rarefaction and disintegrative nuclear changes. The including element may be a single cell of natural size but it is commonly large with multiple nuclei or a heavily chromatic or multilobulated single nucleus (Fig. 6). The included material may consist of a clump of two or three cells or may itself appear multinucleated and even of giant cell type. The suggestion is strong that the included cells have been taken up by hardier individuals to be used as a

direct source of nutrient material, or to be incorporated directly into their own substance. The phenomenon may, in fact, be allied to that of cell fusion.

The question as to whether or not individual cells may under appropriate conditions fuse with other cells of their own type has been a subject of prolonged controversy. Mallory [6] has long championed such power of coalescence as an explanation for the genesis of the foreign body giant cell, an idea now generally accepted. The early suggestion of Krauss [7] that a similar fusion may occur between neoplastic cells has not met with acceptance. It was, however, considered favorably by Steinhaus,[8] who summarizes the literature of a period during which this and allied topics were being actively debated. Haythorn[9] believes that the giant cells studied by Krauss were actually of foreign body type. Fusion of tumor cells was accepted in the earlier work on the culture of malignant tumors of animal and human origin.[10] Cells showing similar fusion were regarded by Lewis [11] as modified macrophages. Carrel and Ebeling [4] explained as degenerative phenomena the fusion forms, as well as the irregular mitoses and other abnormalities seen in cultures prepared by the earlier methods. Similar criticism does not seem to apply to the syncytia appearing in carcinoma cultures. The cell forms of the present material suggest that fusion is actually taking place and that individual tumor cells merge into larger units. Such fusion is believed to offer one explanation for the occurrence of giant cells.

Giant cells appear in nine of the fluids, sometimes in small, but in several cases in large numbers. In only rare cells does the cytoplasmic and nuclear structure suggest the foreign body type. In some cases there are associated multipolar mitotic figures, but again these may be extremely uncommon, so that but one or two may be found in slides containing large numbers of giant cells. Occasionally there are bipolar spindles with apparent excess of chromosome numbers. In fluids with well preserved cells of regular size there may occur rare giant forms reaching diameters of 70 to 80 microns, with a single, round, sharply contoured or slightly fluted nucleus, and with natural balance between nuclear and cytoplasmic volume. In most cases, however, the nuclear volume tends largely to overbalance the cytoplasmic so that relatively great masses of nuclear material are surrounded by the scantiest zone of cytoplasmic substance. The

nuclei may be single or multiple. Marked lobulation is usual and the nuclear subdivisions are heavily dotted with prominent nucleoli. There is often great variation in the chromatin content of the individual nuclear masses or of the irregular subdivisions of the single lobulated nucleus. Many attempts have been made to explain these curious figures. Their variety is bewildering but it is probable that all depend upon a common phenomenon. It is possible that the multiple forms of equal size and chromatin content may result from multipolar mitosis in a nucleus of giant size, but such forms are the exception rather than the rule. On the other hand, multiple forms of unequal size may result from unequal subdivision of chromatic material when division has been accompanied by the presence of an odd number of centrosomes and spindles. Again it is possible that the lobulation and constriction of the single nucleus and the occurrence of separate nuclear masses of unequal size may best be explained by direct nuclear division.[12] The evidence, however, is believed to warrant the contrary suggestion that these bizarre forms result from a successive building up of larger and more deeply chromatic nuclear formations through a gradual process of cell fusion, such fusion involving not only the cytoplasmic material but the nuclear substance as well. While it is usually accepted that the multipolar mitotic figure is associated with the appearance of tumor giant cells, it is not clear which is primary. The question may still be asked as to whether the multiple spindle formation is cause or effect of the hyperchromatic nucleus, whether this nucleus be single, multiple or lobulated. No satisfactory explanation has been given for the original formation of the giant chromatic aggregation. It has usually been regarded by pathologists as a degenerative phenomenon and there is much to support this belief. It has been ascribed also rather vaguely to fluid imbibition or to hypertrophy from unknown causes. Levine [13] suggests diffuse foci of exalted intranuclear growth as the origin of lobulation and is inclined to agree with the zoölogists that the giant size may be an evidence of increased growth energy rather than a forerunner of cell decay. The evidence from tissue culture work favors the long held belief that cell fusion, giant cell formation and atypical mitosis depend in the first instance upon factors unfavorable to cell growth, whether these factors be diminished oxygen tension, changes in hydrogen ion concentration, the accumulation of interfering products of cellular

metabolism, or other influences as yet unknown. The association of degenerative changes is well brought out in a recently studied carcinoma of the breast where thick columns of tumor cells in a medullary carcinoma are suddenly replaced in the marginal zone of an area of gross necrosis by columns made up of cells greatly reduced in number but practically all of giant size with great multiple or lobulated nuclear masses. In the fluids under discussion the number and variety of fusion figures and giant cells appear to vary with the degree of the degenerative changes affecting the various cellular constituents.

Granting that giant cell formation in tumors is a regressive change in the sense of its dependence upon unfavorable growth factors, it may still be accepted that for the individual cell it may constitute actually a protective mechanism. It may then be regarded as the result of a determined struggle for existence on the part of the neoplastic cell and as an indication of its ability to build up a volume of nuclear material far beyond the usual requirements.

Figures believed to represent fusion forms may sometimes appear in early stages as cell clumps with a peripheral enveloping layer of flattened elements one cell thick (Fig. 7). The inner cells appear to fuse gradually and, as this takes place, marked irregularity makes its appearance in the size and chromatin content of the individual nuclear masses of the aggregation. There appears to be a condensation of the nuclear material into one or more segments of a coalescing chain, other segments of which are fading out. There may be separate nuclear formations sharply defined or faintly suggested and more or less devoid of chromatic material (Fig. 3). A final stage is represented by a central single, multiple or lobulated, deeply stained nucleus with many nucleoli and faint cytoplasmic investment, outlined by a prominent surface zone of denser cytoplasm, reticulated or granular and often exhibiting many delicate circumferentially arranged fibrils. The cytoplasm may occasionally contain coarse threads of basic-stained material and not infrequently there are faintly defined shadows suggesting nuclei or nuclear remnants (Fig. 8). Mitosis does not seem to play a part in the formation of these masses. In other cases cell clumps of more open type, with irregularly disposed components, may include cells containing bipolar mitoses of normal chromosome content, or such dividing cells may lie within the cytoplasm of giant cells. Here the nuclear irregu-

larity or giant size appears to have antedated the incorporation of the dividing cell. The fate of such dividing elements cannot be made out. It is possible that their mitosis is abortive and their chromatic material, as well as other constituents, is taken up directly and incorporated by the enveloping cell mass.

Multipolar mitosis appears as commonly in association with giant cells as it does in solid tumors, although, as noted, it may be relatively uncommon or practically absent in the individual specimen. The spindle organization may be well defined, but again it may be highly complex and confusing. The details of centrosphere and spindle structure are often vaguely made out and may even completely escape detection. It is extremely uncommon to find anything suggesting reorganization of the chromosomes into daughter nuclei. There can be little doubt that in at least some of the cells of these fluids attempted multipolar mitosis results in cellular disintegration. It is not uncommon to find cytoplasmic masses permeated by swarms of chromosomes with no regularity of size or arrangement and no suggestion of spindle structure, while the cytoplasmic substance itself is vacuolated and indistinctly stained (Fig. 9). In the cells of tissue cultures Lambert [14] reported that scattered chromosomes of the dividing cell may fail to be incorporated into daughter nuclei, and he observed that dividing cells after reaching the stage of equatorial plate formation might show an arrest of the process and a subsequent break-up of the entire cell substance into small fragments. Cells in multiple mitosis have been most recently described and carefully studied in solid tissues by Levine,[15] who has counted in them as many as 300 chromosomes. He suggests that the chromosomes may divide irrespective of nuclear organization or spindle formation and that, as indicated in the present examples, the process may be followed by disintegration of the chromatic material and possibly of the entire cell. He suggests that the liberated substances may have some influence in stimulating other cells to activity.

Possible answers to some of the many questions suggested by the findings in these fluids may come through study by the newer methods of supravital staining and tissue culture. Our recent attempts in these directions have not been successful, but the effort is being continued.

Summary and Conclusions

1. Neoplastic cells are readily demonstrable in pleural and peritoneal transudates resulting from neoplastic disease of the serosae. They are of interest not only because of their immediate value in clinical diagnosis but also because of the opportunity they afford for cytological study.

2. The tumor cells are believed to proliferate more or less actively within the fluid in which they are suspended. They occur as single cells or as clumps showing a more or less marked tendency toward organoid arrangement.

3. True tumor giant cells occur not infrequently and may be present in large numbers. While their formation probably depends upon unfavorable growth conditions the phenomenon may be for the individual cells a purposeful and protective mechanism.

4. These giant cells appear to result from successive fusions between individual tumor cells, with the gradual building up of great nuclear masses of varying morphology.

5. It is suggested that multipolar mitosis is a result rather than a cause of giant and multiple nucleation of tumor cells.

6. Multipolar mitosis is followed in some cases by cellular disintegration. No figures definitely identifiable as phases of nuclear reconstruction after multipolar mitosis have been seen in the present preparations.

REFERENCES

1. Zemansky, A. P. The examination of fluids for tumor cells. *Am. J. M. Sc.*, 1928, **175**, 489–504.

2. Mandlebaum, F. S. The diagnosis of malignant tumors by paraffin sections of centrifuged exudates. *J. Lab. & Clin. Med.*, 1917, **2**, 580.

3. Ehrlich and Beneke. Quoted by Karp, Ref. 5.

4. Carrel, A., and Ebeling, A. H. The fundamental properties of the fibroblast and the macrophage. *J. Exper. Med.*, 1928, **48**, 105–123.

5. Karp, Herbert. Cytodiagnostik maligner Tumoren aus Punktaten und Sekreten. *Ztschr. f. Krebsforsch.*, 1932, **36**, 579–605.

6. Mallory, F. B. Giant cell sarcoma. *J. Med. Res.*, 1911, **24**, 463–467.

7. Krauss, E. Beiträge zur Riesenzellenbildung in epithelialen Geweben. *Virchows Arch. f. path. Anat.*, 1889, **95**, 249–272.

8. Steinhaus, J. La cellule cancéreuse considérée principalement au point de vue de la genèse et de la nature des inclusions nucléaires et plasmatiques. *J. méd. de Bruxelles*, 1909, **14**, 371, 386, 408, 421, 437, 489, 505, 518, 531, 550.

9. Haythorn, S. R. Multinucleated giant cells. *Arch. Path.*, 1929, **7**, 651–713.

10. Fischer, Albert. Observations on the division of sarcoma cells in vitro. *J. Cancer Research*, 1925, **9**, 71–84.

11. Lewis, W. H. The formation of giant cells in tissue cultures and their similarity to those in tuberculous lesions. *Am. Rev. Tuberc.*, 1927, **15**, 616–628.

12. Mallory, F. B., and Stewart, F. W. Tumor and foreign body giant cells in a fibrosarcoma of the uterus. *Am. J. Path.*, 1927, **3**, 85–89.

13. Levine, M. A comparative cytological study of the neoplasms of animals and plants. *J. Cancer Research*, 1925, **9**, 11–48.

14. Lambert, R. A. Comparative studies upon cancer cells and normal cells. *J. Exper. Med.*, 1913, **17**, 499–510.

15. Levine, M. Studies in the cytology of cancer. *Am. J. Cancer*, 1931, **15** 144–211, 788–834, 1410–1494.

DESCRIPTION OF PLATES

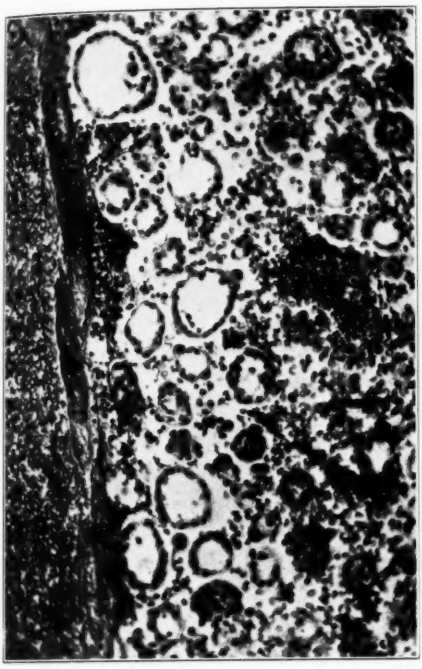
PLATE 115

FIG. 1. Vesicle formation in ascitic fluid. × 150.

FIG. 2. Single cells and cell clumps in ascitic fluid from a case of papillary cystic adenocarcinoma of the ovary implanted widely over the peritoneum. Near the right margin is a hyperchromatic cell in mitosis. Signet formations and cell inclusions are seen. × 150.

FIG. 3. A cell with a single, heavily chromatic giant nucleus and several sharply defined single nuclei, which show varying degrees of chromatolysis. At the top of the illustration is an irregular cell clump with two foci of cytoplasmic and nuclear fusion. × 500.

FIG. 4. At the margin of the (broken) circlet of large cells is a cell in multipolar mitosis. Toward the lower left is a disintegrating cell containing irregularly scattered chromosomes resulting from attempted multipolar division. In upper cell clump two diasters are seen and in the upper portion of the right hand clump two equatorial plates are slightly out of focus. × 300.



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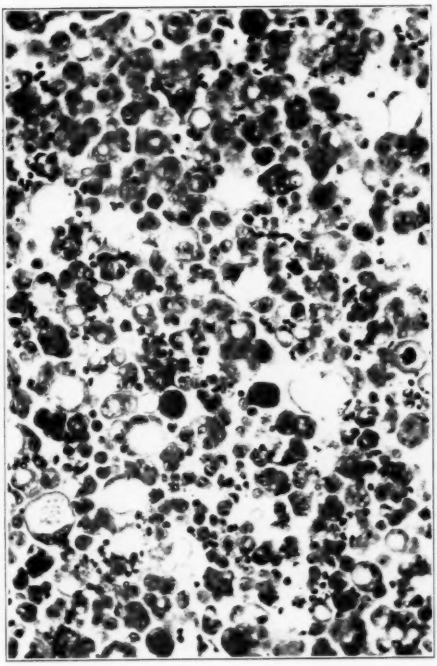

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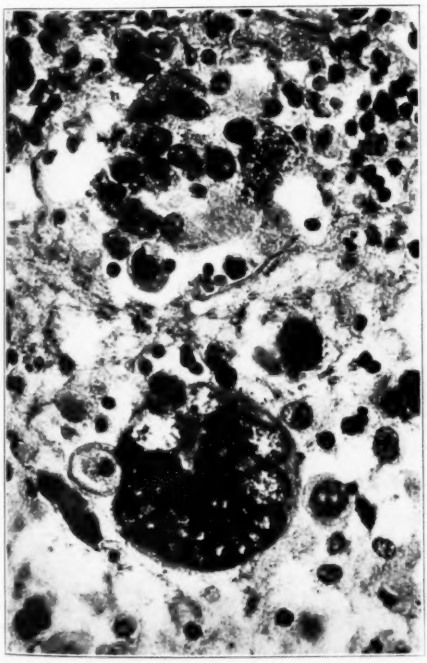

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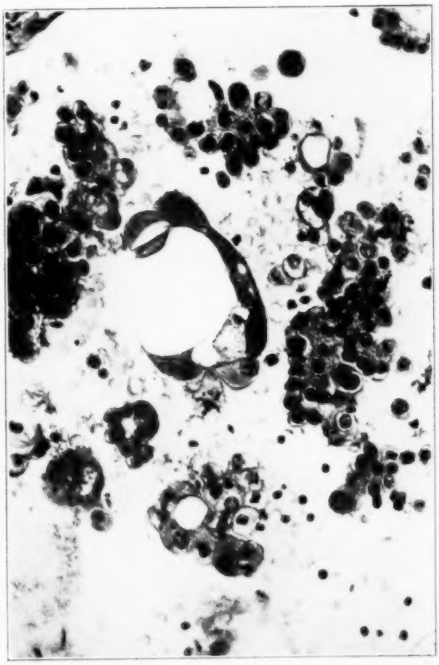


4

Graham

Cancer Cells of Serous Effusions

PLATE 116

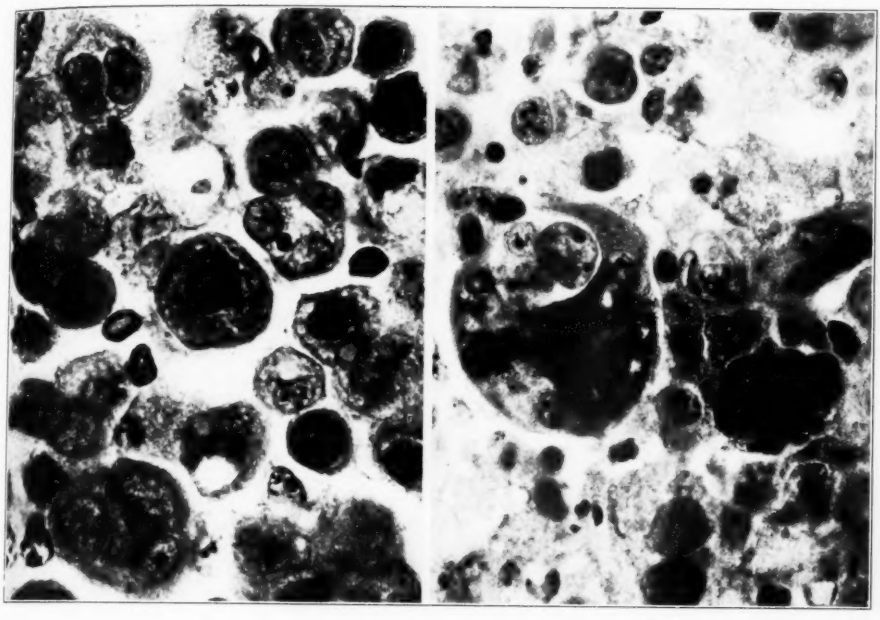
FIG. 5. The central cell contains a large, deeply stained nucleus and three small nuclear bodies, the nuclear membranes of which are blurred and indistinct. The nucleoli are still demonstrable, although not evident at the optical plane shown. The nuclear membranes of one, and probably those of all the smaller masses, are in actual continuity with that of the central mass. The small bodies are believed to be elements in an original cell cluster whose cytoplasmic bodies have fused and whose chromatin content is concentrating in the central nucleus. × 750.

FIG. 6. Tumor giant cell enclosing a multinucleated cell. To the right a giant cell with circlet of nuclear lobules and scanty cytoplasm is seen. × 750.

FIG. 7. A cell clump with flattening and condensation of the marginal cells. The heavily stained nuclear mass is interpreted as an early phase in the growth of a giant nucleus. × 500.

FIG. 8. A smaller cell with nucleus of fusion type and heavy chromatin fragments in its marginal cytoplasm. × 750.

FIG. 9. A giant cell disintegrating after attempted multipolar division. The chromosomes are of unequal size, some of them barely visible in the photograph. A number of minute chromosomes have wandered into the cytoplasmic elongation at one pole of the cell. This cell can be followed through three sections, none of which shows any indication of spindle organization. × 500.



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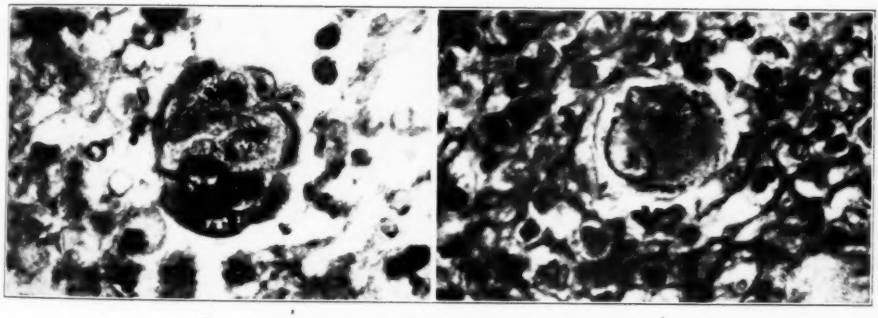

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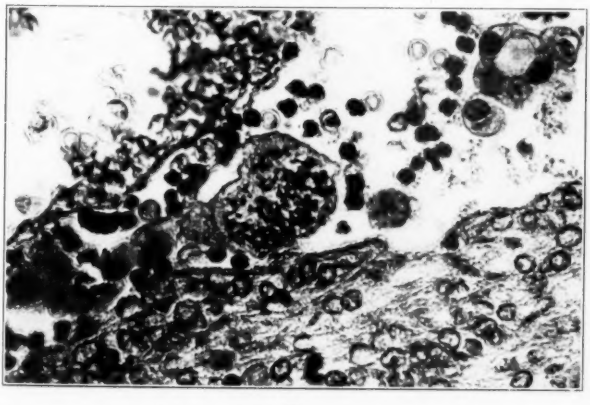

9

THE PERSISTENCE OF TUBERCULOUS INFECTIONS *

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Some of the outstanding facts concerning tuberculosis, which must be appreciated by both the clinician and the pathologist, are its inherent insidiousness and its multiform manifestations. To assert positively that any lesion during life or after death is or is not of tuberculous origin often reveals in the final analysis definite and occasionally humiliating errors. There is no expert, no matter what experience or special training he may have had, who has not been "fooled" by this disease. Such mistakes may cover the entire subject in all its phases, for instance: (1) It is diagnosed as present when it is not. Sometimes this is true even with the finding of tuberculosis-like bacilli in the sputum, or the urine or feces. (2) The patient is assured that tuberculosis is not present when it is. Even macroscopic examination of excised tissues may fail to reveal its hidden presence. Orth [1] demonstrated this fact six years before Koch announced the discovery of the bacillus of tuberculosis, when he found, by the microscope, lesions in grossly normal lymph nodes of animals fed on fodder infected with tuberculous material. (3) The patient or his physician is told that the tuberculosis which he once had has been completely healed. Such a statement can never be made with any great degree of certainty and it is my purpose here to emphasize this fact.

There is nothing new in the idea that tuberculosis in the animal body may be hidden for indefinite periods; also that a tuberculous infection may remain latent or dormant for many years, being revealed only by increase of activity or postmortem examination. Soon after the positive identification of the Koch bacillus workers in laboratories began to demonstrate by animal inoculation the presence of virulent bacilli of tuberculosis in grossly normal lymph nodes removed from apparently non-tuberculous subjects. In 1890 Loomis [2] reported that in 8 of 30 such cases positive results were obtained by animal inoculation. These lymph nodes were not checked by microscopic examination. Pizzini [3] found latent carriers of bacilli

* Received for publication April 21, 1933.

in 42 per cent of his cases. Similar reports were offered by Spengler [4] and Straus.[5] Kälble [6] in 2 of 23 cases infected animals with lymph nodes apparently free from tuberculous lesions, even by microscopic examination. MacFadyen and MacConkey [7] reported similar results. Numerous other students of human pathology have confirmed these experiments, notably Harbitz,[8] and workers in the field of veterinary medicine have published many instances of concealed virulent bacilli of tuberculosis revealed by animal inoculation. Wang [9] reviewed from the literature the total number of such examinations and found that in 357 instances grossly and microscopically normal lymph nodes gave positive results on inoculation into guinea pigs in 12 per cent of cases. In 1905 Weichselbaum and Bartel [10] reviewed the subject and concluded that these organisms might remain latent without multiplication.

Many years before, Kurlow,[11] after experimenting with animals, had expressed the belief that wherever there is a caseous focus there remains for that individual the danger of a further spread of tuberculous autoinfection, and that lesions of tuberculosis can be regarded as healed only when the process shows an old scar or fully completed calcification. Lubarsch [12] tested these conclusions by grinding up partially calcified bronchial lymph nodes and inoculating guinea pigs with them; in a considerable percentage he recovered virulent bacilli of tuberculosis. Rabinowitsch [13] reported results in 5 cases; 4 with fully calcified nodes, and all positive by guinea pig inoculation. Schmitz [14] confirmed these experiments, using partially or wholly calcified nodules from 28 clinical cases, and obtained positive results by guinea pig inoculation in 13 of them. In 2 of these cases he found bacilli of tuberculosis by direct staining methods. Wegelin,[15] by a special method using antiformin, and intensive search, found recognizable bacilli of tuberculosis in 7 of 13 cases in which nodules of tuberculosis were apparently completely healed.

In 1900 Naegeli [16] published the results of his "fine-tooth-combing" at autopsy for evidences of tuberculous infection. As in a successive series of cases his zeal and thoroughness increased, so increased the percentage of positive findings, the four series revealing 75 per cent, 90 per cent, 97 per cent and lastly 98 per cent of adults as harboring gross or histological evidence of tuberculosis. His aphorism "Jeder Erwachsene ist tuberculös" seemed justified. In this study he designated one group of cases as "latent active tuber-

culosis." Of 217 cases in which death was not due to tuberculosis he found the latent active type in 74 (34.1 per cent). These conclusions were the result of anatomical observations and were not controlled by inoculation experiments. Naegeli makes no apology for this anatomical standard but holds that its value rests on the thoroughness and training of the observer.

Birch-Hirschfeld,[17] the previous year, reported that among 826 autopsies performed on subjects who died from accident or acute disease 171 (20.7 per cent) revealed tuberculous lesions. Of these 105 (12.7 per cent) were judged to be healed; 31 (3.8 per cent) were active and well advanced and 35 (4.2 per cent) revealed mildly active or latent processes. As other workers began to publish their statistics it became at once apparent, as would be expected, that the number of latent or comparatively inactive tuberculous lesions increased with the age of the persons examined, just as did the percentage of those showing evidences of infection. Although the percentages of various authors differed over a considerable range the fact just stated continued to stand out in clear-cut prominence. Thus Lubarsch [18] in 1913 found that in 139 bodies of tuberculous subjects between 1 and 16 years of age, only 33 (23.7 per cent) revealed calcification, although in none was the lesion completely healed, while at the age of 40 years, latent or healed lesions were more frequent than the active ones. He also emphasized how careful histological examinations may often reveal activity where grossly none is suspected. Reinhart,[19] in 1917, found in 460 postmortem examinations no lesions in 28 newborn infants, active tuberculosis in 29.16 per cent of 72 children aged less than 16 years, and that of 360 adults 96.38 per cent had signs of the disease. In 63.9 per cent of this latter group the process was regarded as healed. Mönckeberg [20] examined the bodies of 85 soldiers who died in the World War. In 27 of these there were signs of tuberculosis. In Hart's [21] series of 573 soldiers, 196 (34 per cent) had tuberculous lesions, of which 151 (26.8 per cent) were quiescent.

In this same year, 1917, Opie,[22] using X-ray plates to identify the less readily distinguishable nodules in lungs and hilum nodes, discovered that whereas about 8.3 per cent of infants aged up to 2 years were tuberculous at death, in from 2 to 10 years this percentage increased to 44, from 10 to 18 years to 66.7 and beyond that period 100 per cent of bodies (50) revealed lesions of tuberculosis.

In a later study, 1927, Opie and Aronson [23] by guinea pig inoculation endeavored to ascertain what proportion of apparently healed lesions contained living bacilli of tuberculosis. Material from 169 bodies was thus examined and gave positive results in 52 cases (30 per cent). When they selected pulmonary tissues, which were apparently free from tuberculosis (although signs of the disease might be present in other portions), in 33 cases 15 (45+ per cent) gave positive results.

In spite of rather wide variations in percentages it is quite clear that almost every adult (at least in the past generation) had been at one time infected with virulent bacilli of tuberculosis, and that many of them at the time of death still harbored infective organisms, even though their lesions might have reached a quiescent stage. I have purposely omitted references to clinical reports on this subject. Various tuberculin tests, studies of heredity, and records of recurrences of the disease, only confirm what Fishberg [24] denominates as "the frightful tuberculization of humanity."

My own experience closely parallels that of other students of tuberculosis, and for many years my attention has been focused on those cases demonstrating the latent or dormant characteristics of the disease. This emphasis has seemed all the more justifiable, not only because of the relative frequency of such cases, but also because the recent apparent subsidence of the virulence of the tuberculosis pandemic has led to extravagant statements about its curability, thereby tending to foster a spirit of blind optimism, which the facts do not warrant. Thus Jaquerod [25] stated that after one year devoted to a "clinical cure of pulmonary tuberculosis" and another twelve months to "confirming and consolidating this result . . . the patient will be in a condition to return to a normal life without any risk. . . . Once this period is past the healing can be considered as definitive."

In the study, the results of which I wish to report, consideration has been given to the data of family and clinical history, physical examination and postmortem examination, both gross and microscopic. No attempt was made to verify the presence of the bacillus of tuberculosis either by staining or animal inoculation. The final criteria for the diagnosis of active tuberculosis rested on histological evidences of activity on the part of the cells in the tuberculous area, such as foci of connective tissue proliferation, giant cells and agglom-

eration of lymphocytes. Such standards do not entirely eliminate the possibility of errors, particularly as the lesions of silicosis often simulate a chronic tuberculous inflammation, but such sins of commission are more than outweighed by the sins of omission. Undoubtedly more painstaking methods would reveal a much larger percentage of lesions containing viable, virulent bacilli (Figs. 1–5).

A few cases are presented in abstract to illustrate the data employed in the tabulation:

CASE 1. A woman, aged 58 years, died suddenly from coronary occlusion. At the age of 12 she had suffered from tuberculosis of the spine. During the intervening forty-six years there had never appeared any manifestations of tuberculosis. However, the lymph nodes at the roots of the lungs were the site of progressive chronic tuberculous disease marked by the presence of fresh tubercles, giant cells and necrosis.

CASE 2. A man, aged 24 years, was killed suddenly in an automobile accident. He was a farmer and had always been in good health. There was no family history of tuberculosis. His physical development and condition was almost perfect. In spite of these facts examination of several enlarged lymph nodes at the hilum of the lungs revealed chronic progressive, well advanced tuberculosis.

CASE 3. An elderly man, aged 82 years, who had never been ill, and had a son living and well, on clinical examination presented no evidence of tuberculosis. Death was due to coronary sclerosis and infarction of the myocardium. In the lung were found old tuberculous lesions which revealed evidences of histological activity.

CASE 4. A woman, aged 64 years, died from bronchopneumonia following an operation for trifacial neuralgia. There was no family or personal history or clinical evidence of tuberculosis. A son was living and well. At autopsy a healed lesion was found in the lungs and active tuberculous lymphadenitis in the aortic and hilum nodes.

It was such occurrences as these that have led me to review the entire series of autopsies performed at The Mayo Clinic over a period of six years (1926–1931 inclusive), in order to determine the relative incidence of the various classes of tuberculous processes. The results represent the "run" of a moderately efficient mill.

During these six years approximately 3306 postmortem examinations revealed an incidence of some form of tuberculous lesion in 2064 (62.43 per cent). Of this group in 89 cases tuberculosis was either a principal or contributing cause of death, and in 1725 cases the tissues examined contained apparently entirely healed tuberculous processes. In Table I are arranged according to decades those cases in which were found active lesions unrecognized clinically. They total 134 (4.05 per cent) of the total examinations.

As previously admitted more detailed examinations or animal inoculations undoubtedly would have materially raised this percentage. Many lesions were pronounced healed because clear evidences of histological activity were not present. Previous workers have

TABLE I

The Incidence of Active Tuberculosis (Unsuspected Clinically) Found at Postmortem Examination

Age in years	No. of examinations	Cases of tuberculosis	Per cent
1 to 9	136	4	2.94
10 to 19	134	9	6.71
20 to 29	219	15	6.84
30 to 39	396	15	3.78
40 to 49	598	19	3.17
50 to 59	783	35	4.47
60 to 69	726	29	3.99
70 to 79	267	4	1.49
80 to 89	43	4	9.30
90 to 99	4	..	..
Total	3306	134	4.05

demonstrated that a certain percentage of these lesions will reveal virulent organisms. Even so, the number is sufficiently impressive to reëmphasize the extreme tenacity of the tuberculous infection.

CONCLUSIONS

From this and the other reported studies the following conclusions would appear justifiable:

1. Tuberculous infections may occur and pursue their entire course without demonstrable clinical phenomena, that is, without attracting attention of patient or physician to their presence.

2. Recognized tuberculous infections may subside and be re-

garded throughout remaining life as healed and still remain continuously active.

3. Apparently healed tuberculous lesions may become clinically active after varying intervals.

4. No form of physical examination can assure any individual that he or she does not harbor the menace of active tuberculous infection.

5. The safest rule for physicians and patients alike is to regard tuberculosis as possessing an ever present potentiality for becoming active. One can almost say: "Once infected, always infected."

REFERENCES

1. Orth, J. Experimentelle Untersuchungen über Futterungstuberculose. *Virchows Arch. f. path. Anat.*, 1879, **76**, 217–242.
2. Loomis, H. P. Some facts in the etiology of tuberculosis, evidenced by thirty autopsies and experiments upon animals. *M. Rec.*, 1890, **38**, 689–698.
3. Pizzini, L. Tuberkelbacillen in den Lymphdrüsen Nichttuberkulöser. *Ztschr. f. klin. Med.*, 1892, **21**, 329–342.
4. Spengler, C. Zur Bronchialdrüsentuberculose der Kinder. *Ztschr. f. Hyg. u. Infectionskrankh.*, 1893, **13**, 347–356.
5. Straus, I. Sur la présence du bacille de la tuberculose dans les cavités nasales de l'homme sain. *Rev. de la tuberc.*, 1894, **2**, 198–204.
6. Kälble, Johannes. Untersuchungen über den Keimgehalt normaler Bronchiallymphdrüsen. *München med. Wchnschr.*, 1899, **46**, 622–625.
7. MacFadyen, Allan, and MacConkey, Alfred. An experimental examination of mesenteric glands, tonsils and adenoids. *Brit. M. J.*, 1903, **2**, 129–130.
8. Harbitz, F. Untersuchungen über die Häufigkeit, Lokalisation und Ausbreitungswege der Tuberkulose, insbesondere mit Berücksichtigung ihres Sitzes in den Lymphdrüsen und ihres Vorkommens im Kindesalter. J. Dybward, Kristiana, 1905, 166.
9. Wang, C. Y. An experimental study of latent tuberculosis. *Lancet*, 1916, **2**, 417–419.
10. Weichselbaum, A., and Bartel, Julius. Zur Frage der Latenz der Tuberkulose. *Wien. klin. Wchnschr.*, 1905, **18**, 241–244.
11. Kurlow. Ueber die Heilbarkeit der Lungentuberkulose. *Deutsches Arch. f. klin. Med.*, 1889, **44**, 437–459.
12. Lubarsch, O. Quoted by Rabinowitsch. Zur vergleichenden Pathologie der Tuberkulose. *Deutsche med. Wchnschr.*, 1908, **34**, Pt. 2, 1921–1923.
13. Rabinowitsch, Lydia. Zur Frage latenter Tuberkelbacillen. *Berl. klin. Wchnschr.*, 1907, **44**, 35–39.

14. Schmitz, E. Experimentelle Untersuchungen über die Virulenz latenter tuberkulöser Herde beim Menschen, Rind und Schwein. *Frankfurt. Ztschr. f. Path.*, 1909, **3**, 88–169.

15. Wegelin, Carl. Ueber den Tuberkelbazillengehalt verkalkter Herde. *Cor.-Bl. f. schweiz. Aerzte*, 1910, **40**, 913–921.

16. Naegeli, Otto. Ueber Häufigkeit, Localisation und Ausheilung der Tuberculose. *Virchows Arch. f. path. Anat.*, 1900, **160**, 426–472.

17. Birch-Hirschfeld, F. V. Ueber den Sitz und die Entwicklung der primären Lungentuberkulose. *Deutsches Arch. f. klin. Med.*, 1899, **64**, 58–128.

18. Lubarsch, O. Beiträge zur Pathologie der Tuberkulose. *Virchows Arch. f. path. Anat.*, 1913, **213**, 417–427.

19. Reinhart, A. Anatomische Untersuchungen über die Häufigkeit der Tuberkulose. *Cor.-Bl. f. schweiz. Aerzte*, 1917, **47**, 1153–1162.

20. Mönckeberg, J. G. Tuberkulosebefunde bei Obduktionen von Kombattanten. *Ztschr. f. Tuberk.*, 1915, **24**, 33–38.

21. Hart, C. Pathologisch-anatomische Beobachtungen über die Tuberkulose am während des Krieges sezierten Soldatenmaterial. *Ztschr. f. Tuberk.*, 1919–20, **31**, 129–138.

22. Opie, E. L. The focal pulmonary tuberculosis of children and adults. *J. Exper. Med.*, 1917, **25**, 855–876.

23. Opie, E. L., and Aronson, J. D. Tubercle bacilli in latent tuberculous lesions and in lung tissue without tuberculous lesions. *Arch. Path.*, 1927, **4**, 1–21.

24. Fishberg, Maurice. Pulmonary Tuberculosis. Lea & Febiger, Philadelphia, 1922, Ed. 3, 68.

25. Jaquerod, Marc. The Natural Processes of Healing in Pulmonary Tuberculosis. Baillière, Tindall & Cox, London, 1926, 107.

DESCRIPTION OF PLATE

Plate 117

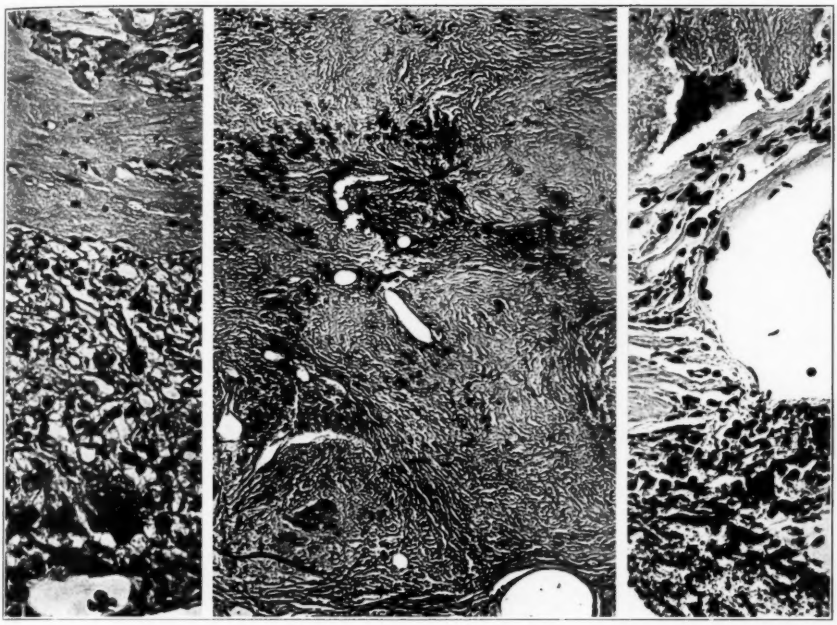
Fig. 1. Hilum node from a man aged 74 years. Chronic tuberculosis. × 315.

Fig. 2. Hilum node from a man aged 60 years. Chronic tuberculosis. × 95.

Fig. 3. Lung from a man aged 69 years. Chronic tuberculosis. Minimal histological signs of activity. × 275.

Fig. 4. Lung; same case as that shown in Fig. 2. Active chronic tuberculosis. × 75.

Fig. 5. Bronchus; same case as that shown in Fig. 2. Chronic tuberculosis. × 50.



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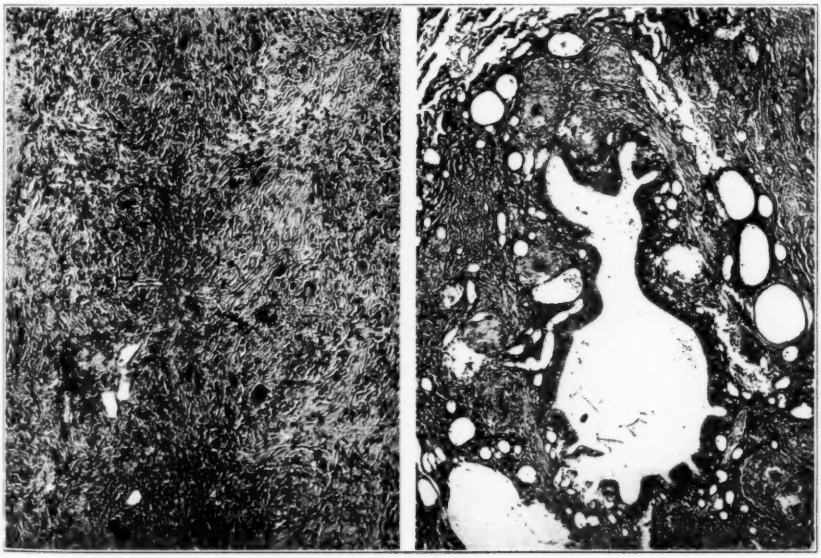

4 5

Robertson Persistence of Tuberculous Infections

RHEUMATIC PERITONITIS *

LAWRENCE J. RHEA, M.D.

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There are references to abdominal symptoms in "rheumatism" as long ago as 1635 when Ballonius,[1] in a discussion on the differential diagnosis between rheumatism and gout, referred to diarrhea, which occasionally accompanied rheumatism. He felt that this was beneficial, as the "humeurs" were, by this means, discharged from the body. At that time, and for a good while afterward, the term "rheumatism" was used in a general sense and was applied to such a large and varied group of diseases that one cannot be sure whether or not those to which Ballonius referred were rheumatic fever as we understand the disease today.

In 1752 Huxham,[2] in an article in which he discussed the localization of abdominal symptoms, referred to these as preceding rheumatic fever as we now know it. Since then there has been a sustained but fluctuating interest in these manifestations.

In recent times there has been not only a renewed and increasing interest in these symptoms, but some of these have been interpreted as resulting from rheumatic lesions in the peritoneal tissues. In an article on abdominal symptoms in acute rheumatic fever Giraldi,[3] in 1930, discussed them under three headings: (1) the digestive group, (2) the pseudoappendiceal group, and (3) the peritoneal group.

There are several cases referred to [4-7] in which acute abdominal symptoms developed during the course of, or were followed by, rheumatic fever. In some of the cases no lesion was found; in others a variety of conditions such as clear fluid, simple hyperemia with very little exudate, or fibrinous, purulent or even hemorrhagic exudate occurred. These have been interpreted as peritoneal reactions to the causative agent of rheumatic fever.

There is no unanimity in regard to the number of cases in which the involvement of the peritoneum is thought to be responsible for symptoms; in fact positive proof of peritoneal involvement is limited. This is probably due to the fact that the peritoneal lesions may

* Received for publication June 15, 1933.

not be visible to the naked eye and, more important than this, until comparatively recently rheumatic fever was looked upon as a disease that involved the heart and joints alone, and detailed microscopic study was largely limited to these organs.

Our conception of rheumatic fever today is much broader than it has been in the past. The disease is no longer thought to involve the heart and joints alone. We look upon it as a general infection that involves the body as a whole and results in a special kind of cellular reaction often with gross changes in various organs of the body. The cellular reaction in rheumatic fever is as specific for that disease as that found in tuberculosis. Like the microscopic lesion in tuberculosis that in rheumatic fever is not always the same in detail. The cellular reaction in tuberculosis is not that of the classical tubercle alone; it may be, and often is, much more diffuse. Neither does the cellular reaction of rheumatic fever result only in a typical Aschoff body; the lesions may be diffuse and yet be specific.

It has been recognized for a long time that there occurs a specific rheumatic lesion in the pericardium and in the pleura, and the signs and symptoms dependent on them can be recognized clinically. The peritoneum is, in general, similar to the pleura and pericardium and one might theoretically expect rheumatic lesions to occur there. If they occur, some of them should lead to clinical signs and symptoms.

In a comparatively large number of postmortem examinations on individuals dying of rheumatic fever at the Montreal General Hospital, but one has shown rheumatic lesions in the peritoneum. Following is a brief summary of this case.

Report of Case

Clinical History: A. B., male, aged 25 years, was admitted to the Montreal General Hospital with abdominal cramps and aching pains in the front of the chest, radiating to the back. Palpitation of the heart, dyspnea and a feeling of suffocation were present. There was a history of constipation, anorexia and loss of weight. The patient had had three attacks of rheumatic fever, one at the age of 4 years, one at 17 years and one in his 25th year. For some years he had had the symptoms resulting from an enlarged and incompetent heart. The winter before his admission to the hospital he had had his third attack of rheumatic fever, which had confined him to bed for a month. While in the hospital he had, in addition to the clinical signs and symptoms associated with an enlarged and incompetent heart, an abdomen that was rounded and a little distended, with abdominal muscles that were resistant. The liver and spleen were not palpable. There was slight, fixed dullness posteriorly. With rest in bed and proper medi-

cation most of the signs and symptoms partly or wholly disappeared. The abdomen, however, remained distended. The liver finally reached three fingers' breadth below the costal margin in the midclavicular line. The heart became more incompetent. During the last days of his life the patient was restless and complained of generalized abdominal pain. He died four months after admission to the hospital.

Clinical Diagnoses: Rheumatic pancarditis, chronic aortic valvular disease with marked insufficiency, mitral valve insufficiency, cardiac hypertrophy (*cor bovinum*), adhesive pericarditis, chronic passive congestion of the lungs, liver and kidneys, and rheumatic peritonitis.

Postmortem Examination

The heart, which weighed 1150 gm., showed the changes that are found in recurrent rheumatic fever, and there was evidence of marked incompetence. The pericardium seemed to be completely obliterated by fibrous adhesions, in which a few discrete nodules 5 to 6 mm. in diameter were palpable. The myocardium contained a number of irregularly distributed, pale grayish foci 1 mm. in diameter. On all four valves a row of small, quite firmly attached vegetations were present, with least involvement of the pulmonary valve. In addition, old rheumatic lesions were present on the mitral and aortic valves. The pleurae were thickened and united in places by old adhesions. In some areas there were many petechiae on the parietal pleura. The lungs and spleen showed chronic passive congestion. The peritoneal cavity contained approximately 500 cc. of clear, straw-colored fluid. Anteriorly the parietal layer was smooth and glistening, while laterally and posteriorly there were plaque-like areas palpable in the subperitoneal tissues. The peritoneum in these regions showed the general characteristics of acute inflammation; it was dull, hyperemic and fine granules of fibrin were adherent. Similar lesions were seen in the peritoneum opposite the upper pole of the spleen. The walls of the intestines and the mesentery were edematous and congested.

Microscopic Examination

Heart: On microscopic examination the myocardium shows widespread areas of old, healed, rheumatic lesions. There are also numerous, typical, active Aschoff bodies, which are particularly abundant in the epicardium. One area in the endocardium of the left auricle shows an acute rheumatic lesion. The pale areas in the myocardium,

noted in the gross, prove to be rheumatic lesions. There is marked scarring and contraction of the mitral valve and the lesions of active rheumatic fever are present. Typical old and recent rheumatic lesions are found in the pericardium. In the connective tissue uniting the two layers of the pericardium there are numerous small spaces lined with mesothelial cells. The nodular areas seen in the gross are rheumatic lesions.

Aorta: In the first part of the aorta the periaortic tissues show the cellular changes characteristic of rheumatic fever. Sections of the parietal pleura, including the intercostal muscles and underlying fat, also contain foci of active rheumatic fever in the connective tissue.

Peritoneum: Blocks of tissue were removed from numerous places in the peritoneum. They were all cut so as to include the peritoneum and its underlying tissues. The great majority of these sections show only congestion of blood vessels, with marked edema and irregularly distributed monocytes. In other sections, including those taken from the lesions that were seen in gross, there are various stages of an acute inflammatory reaction. On the whole they are similar to the lesions noted in the heart. There are collections of monocytes, particularly about the blood vessels. Round and linear areas infiltrated with monocytes are also present. Among these monocytes there is an occasional lymphocyte, but no polymorphonuclear leukocytes are found. One of the sections taken from the peritoneal tissue includes a part of the psoas muscle. This section shows in the edematous, subperitoneal connective tissue and the psoas sheath a diffuse, acute inflammatory exudate, which occurs as linear bands of closely packed, elongated and round cells with deeply staining nuclei, and opaque, homogeneous, slightly acidophilic cytoplasm. Here and there are multinucleated cells. No necrosis is present. A few blood vessels show hyperplasia of the endothelial lining.

Conclusions

Except for the fact that the peritoneal lesions are diffuse, rather than focal, and sharply outlined, they are similar in every way to the so-called Aschoff bodies found in the heart in this case and in other cases of rheumatic fever.

We believe that these lesions are specific, resulting from the action upon the peritoneum of the causative agent of the disease.

It seems probable that the involvement of the peritoneum in rheumatic fever is more common than is generally thought. If careful microscopic examination is made of tissues from various regions of the peritoneum in instances of rheumatic fever, especially in the severe acute cases, the specific lesion may be found to be fairly common.

REFERENCES

1. Ballonius, G. Oeuvres posthumes, 1635.
2. Huxham, J. Observationum de aëre et morbis epidemicis volumen alterum; ab anni nimirum initio 1738 ad exitum esque 1748. J. Hinton, London, 1752.
3. Giraldi, J. J. J. Abdominal symptoms in acute rheumatism. *Arch. Dis. Childhood*, 1930, **5**, 379–386.
4. Paul, J. R. Localized peritonitis in rheumatic fever: a case report. *Bull. Ayer Clin. Lab.*, 1930, **12**, 9–15.
5. Wood, F. C., and Eliason, E. L. Rheumatic peritonitis. *Am. J. M. Sc.*, 1931, **181**, 482–494.
6. Tallerman, K. H. Abdominal manifestations in rheumatism. *Brit. M. J.*, 1931, **2**, 844–845.
7. Macdonald, R. A. Streptococcal allergy in acute rheumatic infection. *Arch. Dis. Childhood*, 1930, **5**, 60–72.

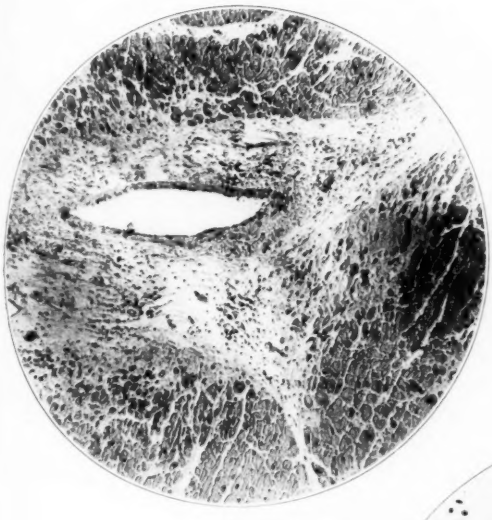
DESCRIPTION OF PLATE

PLATE 118

FIG. 1. Section of heart showing old and recent lesions of rheumatic fever. Several Aschoff bodies are seen in the perivascular connective tissue.

FIG. 2. Retroperitoneal lesion in rheumatic fever. Section through retroperitoneal tissue. The subperitoneal tissue shows edema and infiltration with monocytes. Part of the psoas muscle is seen.

FIG. 3. Peritoneal lesions in rheumatic fever. The subperitoneal connective tissue shows dilatation of its blood vessels, edema and a linear area of cellular exudate. The cells are nearly all monocytes.



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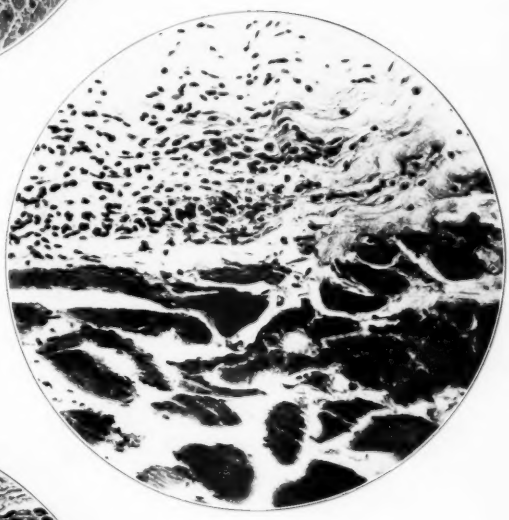

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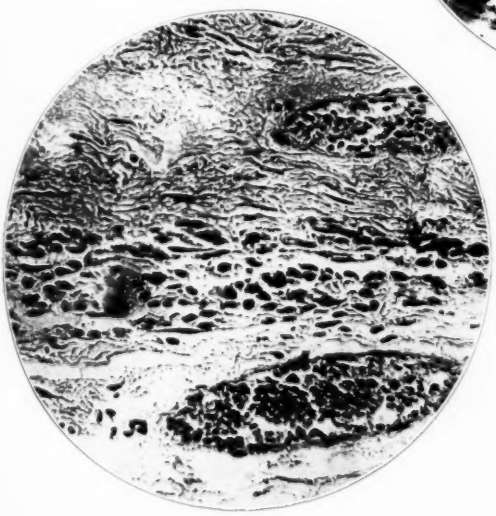

3

Rhea

Rheumatic Peritonitis

NODULAR LESIONS OF THE PERITONEUM *

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Some years ago a nodule was sent to the laboratory for frozen section verification of the gross diagnosis of tuberculous peritonitis. The diagnosis of "foreign body granuloma encapsulating food particles" was returned at once. The history subsequently sent to the laboratory revealed the fact that the patient had had a previous operation for a perforated ulcer. This case, which is given in some detail later, served to fix my interest on the more unusual types of nodules of the peritoneum discovered at operation.

Most of the articles that discuss such nodules deal with isolated case reports of the respective conditions that may produce such lesions. Textbook descriptions are for the most part rather incomplete and sketchy. Kaufmann [1] has a good chapter on lesions of the peritoneum and Hertzler [2] deals with a number of peritoneal lesions under the name of "pseudotuberculosis." Since this subject as a whole has not received much attention in the literature I have undertaken the preparation of an outline of its possibilities and will use it as an index of the subject matter to be discussed. Our own cases are reviewed and they will be used, so far as possible, for illustration.

OUTLINE OF NODULAR LESIONS OF THE PERITONEUM AND OMENTUM

1. Tuberculosis.
2. Pseudotuberculosis (bacillary).
3. Syphilis.
4. Fungus infections.
5. Foreign body granulomas due to:
   (*a*) Animal parasites.
   (*b*) Extravasated blood.
   (*c*) Gelatinous substances — including ruptured pseudomucinous cysts, mucoceles of the appendix and extraneous gelatinous substances.

* Received for publication April 1, 1933.

(*d*) Oils and free fats — including extraneous lipoidol substances, ruptured dermoid cysts and encapsulated foci of fat necrosis.

(*e*) Extruded stomach and intestinal contents.

(*f*) Extraneous foreign bodies, such as sutures, threads, bits of sponge, and so on.

(*g*) Neoplasms, such as carcinomas, sarcomas, melanosarcomas, nodules from cases of Hodgkin's disease, and so on.

Since this discussion is to deal with the more unusual forms of peritoneal nodules I am omitting the subjects of tuberculosis and neoplastic nodules and will take up the other conditions in the order given.

Pseudotuberculosis (Bacillary)

The term pseudotuberculosis has been applied by Hertzler and others to this whole group of lesions. There are two objections to this wider application: (1) the term was used by earlier writers in a specific sense to describe a group of bacillary infections which produced lesions resembling anatomical tubercles, and (2) the lesions are more commonly mistaken clinically for newgrowths, rather than for tuberculosis.

In its specific sense pseudotuberculosis includes a number of spontaneous infections which occur commonly in animals and occasionally in man. Among the organisms that have been reported as isolated from these conditions are *Bacillus pseudotuberculosis* of Pfeiffer, *Bacillus pseudotuberculosis rodentium*, *Bacillus pestis caviae*, *Bacillus abortus* (Bang), *Bacillus melitensis* and *Bacterium tularense*.

Tularemia is the only one of this group of infections that has occurred in our material. The sections from four human cases, including one complete autopsy performed in our laboratory, have been studied and the resemblance of the lesions to anatomical tubercles has been found to be striking. In the absence of the history it is doubtful if anything other than active tuberculosis in the stage of early caseation would have been suspected. Reimann and Rose [3] recently reviewed a case of pseudotuberculosis due to a *Bacillus pestis* infection, reported some years ago by Roman, and suggested that it probably was tularemia. The authors mentioned the absence of giant cells as a differential point between tularemia and tuber-

culosis. The point is not to be relied upon, since there were numerous typical Langhans giant cells in many of the tularemic lesions of our series.

Syphilis

There are numerous statements that gummas of the peritoneum and omentum may occur, but I have found no descriptions of such lesions. In almost twenty years the diagnosis of syphilis of the omentum occurs only once in our files. On account of its rarity I am citing the case.

A patient admitted for acute cholecystitis was found at operation to have several white nodules in the liver. The omentum appeared flecked with pinkish white nodules.

Microscopically the material from the liver was definitely gummatous. The omentum was traversed by thick-walled vessels about which there were wide collections of lymphocytes and eosinophiles. Between the vessels the omentum was clear, save at one point where a nodule of ordinary granulation tissue was present. Near two of the vessels were minute foci of necrosis, one of which was circumscribed and contained numerous foam cells. The other lesion was diffuse, poorly defined and surrounded by a mononuclear and lymphocytic exudate. With the exception of the localization of the process about the vessels there was nothing characteristic of syphilis and the diagnosis depended upon the presence of the gummas in the liver and on a positive Wassermann reaction.

Fungus Infections

Primary fungus infections of the peritoneum appear to be rare. Actinomycosis [1] (see page 854) secondary to other lesions due to this organism has been described. Our experience has been limited to animal experiments. Monilia and Trichophyta injected experimentally have produced typical pseudotubercles in the omentum of guinea pigs and rabbits. Such tubercles showed little tendency to undergo caseation necrosis, though they regularly contained typical Langhans giant cells. *Aspergillus fumigatus* cultures from a human source, with which we have been experimenting for some months, sometimes produce pseudotubercles and at other times form abscesses closely resembling actinomycosis, and can be differ-

entiated from the latter only by oil immersion examination of the stained organisms. From our experiments it seems that almost any fungus pathogenic for man is capable of forming secondary lesions in the omentum which may grossly resemble tuberculosis.

Foreign Body Granulomas

By far the largest number of our cases of peritoneal nodules were foreign body granulomas. Although we have not had examples of the items outlined we have drawn on the literature in order to make the topical discussion complete.

(*a*) *Foreign Body Granulomas Due to Animal Parasites:* In some parts of the world parasites are common causes of nodular lesions of the peritoneum and several cases have been reported from this country. *Entameba histolytica* (Gunn and Howard [4]) may cause granulomas of the bowel resembling carcinoma, with extensions to the peritoneum leading to fibrous encapsulation and foreign body giant cell formation. *Oxyuris vermicularis* (Goodale and Krischner [5]) may become encysted in the mesentery and resemble tubercles. Ascaris ova [1] and bits of chitinous membrane from echinococcus cysts (Del Campo [6]) may become free in the peritoneal cavity and act as centers for foreign body granulomas. Hutchison [7] described peritoneal granulomas fourteen times and tubercle-like granulomas of the epiploic appendages twice in sixty-five cases of *Bilharzia* infestation reported by him from Tanta, Egypt. Several years before this report he sent me some *Bilharzia* material and I studied the granulomas at first hand. It was the best material for the study of foreign body giant cell formation I have observed. The ova had worked their way into the stroma of the fat tissue and set up inflammatory processes. Many embryos had died and leukocytes had entered the shells and digested the embryonal débris. Mononuclear cells were gathered about the outer surfaces of empty eggs, had become granular and fused into large giant cells. The ova shells themselves acted as small bottles or flasks into which the mononuclear phagocytes were seen to be passing in considerable numbers. At one end of such a flask occasionally might be seen separate and distinct phagocytes, while at the other end were large, fused, syncytial masses filling that part of the cavity. The indications from the study of the *Bilharzia* material pointed toward fusion as the mode of formation of giant

cells, there being no mitoses and no evidences of direct multiplication of the nuclei of phagocytes.

(*b*) *Foreign Body Granulomas about Extravasated Blood:* The intraperitoneal hemorrhages that appear most likely to produce foreign body granulomas are those that follow the rupture of graafian follicles, corpora lutea and tubal pregnancies, or the repeated, small, bloody extravasations that sometimes accompany the backward flow of menstrual blood through the tubes in instances of atresia of the cervix. Sampson [8] has pointed out that bits of displaced endometrium may exude blood during menstrual periods.

In our series there were six cases in which the tubes, broad ligaments, dependent peritoneal surfaces and mesoappendices were covered with small, slightly raised, yellowish brown nodules. The pathogenesis of the lesions was not entirely clear. They occurred five times in association with long standing tubal infections, two of which were diagnosed tuberculosis at operation, and once in a patient who had had a spontaneous abortion three weeks before a laparotomy was performed. A tubal abortion of a twin pregnancy was considered before operation, though no evidences of it were found when the abdomen was opened and no explanation of the multiple hemorrhagic spots was obtained.

Microscopically the nodules in all of the cases were similar and consisted of masses of phagocytes filled with yellowish brown pigment. Among these phagocytes were multinucleated giant cells grouped about crystalline deposits. Careful search was made in each instance for endometrial transplants but none was found. The nodules in this series were light yellowish to pale brown in color. Several textbooks of pathology mention the fact that blood spots in the abdomen may take up sulphuretted hydrogen (pseudomelanosis), which von Gierke [9] states changes them to a grayish green or black color.

(*c*) *Foreign Body Granulomas about Gelatinous Substances:* That gelatinous substances freed through the rupture of pseudomucinous cysts of the ovaries and of mucoceles of the appendix may produce foreign body granulomas of the peritoneum is well known. In addition to examples of these we have had a case of a mucocele of Meckel's diverticulum with peritoneal extensions, and one of multiple granulomas in a young woman in whom the source of the material was not discovered.

I have reviewed our records of eighty-two large ovarian cysts, exclusive of dermoids, and have found only six of these which at operation showed peritoneal extensions or nodules. Three of them were malignant and the sections both from the cysts and from the nodules were carcinomatous. Another cyst appeared to be a ruptured simple pseudomucinous cyst, the transplants of which contained secreting epithelium producing much gelatinous material. This lesion appeared both clinically and pathologically to be the condition generally diagnosed as *pseudomyxoma peritonei*. The patient made a complete recovery and was entirely well when last interviewed five and one-half years after the operation. Two other cases showed simple pseudomucinous cysts and the extensions from them were essentially foreign body granulomas, though there were small islands of epithelium with colloid in one. The peculiar finding in these nodules was the unusually large amount of fibrous connective tissue produced, in comparison to the small amounts of colloid, as well as the small size of the giant cells.

We had one instance of a ruptured mucocele of the appendix though the extrusion of the gelatinous material appeared to have been too recent to permit granuloma formation. A patient had been operated upon for a ruptured appendix which was found to be walled off in the ileocecal fossa. The appendix was not removed, a drain was inserted and recovery followed eventually. Six months later the patient was readmitted with nephrolithiasis. A nephrectomy was attempted but owing to the poor condition of the patient it was abandoned. Two days later he died. At autopsy several large kidney stones were found. The omentum was adherent in the ileocecal fossa and beneath it was a yellowish green-colored gelatinous material, which bathed the cecum and stained the surrounding peritoneal surfaces. The material was exuding from a small dilated saccule, the wall of which was thinned out and proved to be the remnant of the old appendix. A double handful of the mucoid material had gravitated to the pelvis where it was lying free behind the bladder.

In reviewing a series of cases of *pseudomyxoma peritonei* associated with appendiceal mucoceles, and in reporting a case of his own, Dieker [10] made the following points. Mucocele of the appendix follows obliteration of the lumen, usually in the proximal half. This is followed by the continued secretion of the appendiceal mucosa in the

encysted distal portion. As the process continues the muscularis becomes stretched to such an extent that the fibers may become separated and the contents escape from small openings formed without actual rupture. As the lumen is no longer open, contamination by feces from the intestinal canal no longer occurs and the enclosed pseudomucin becomes sterile and acts only as a chemical irritant. The gelatinous secretion has at least one fraction which is insoluble in body fluids and this becomes organized with foreign body encapsulation.

If epithelial transplants occur at the time of the escape of the gelatinous material the foci of pseudomucin production may become widespread and the condition known as *pseudomyxoma peritonei* result. Frank [11] cites an instance of this disease following the rupture of a pseudomucinous cyst of the ovary in which 350 pounds of gelatinous material was removed from a patient in twelve operations.

During an exploratory operation on a patient with a large abdominal mass lying to the left of the umbilicus a colloid cyst of Meckel's diverticulum, about 8 cm. in diameter, was found. At the junction of the diverticulum and the intestine there was a firm leiomyoma about 5 cm. in diameter. The omentum, mesentery, and neighboring peritoneum were covered with thin-walled cysts. A clinical diagnosis of colloid carcinoma was made. The pathological examination revealed a mucocele of Meckel's diverticulum without evidence of malignancy or rupture, a benign leiomyoma and foreign body extension cysts of the peritoneum filled with pseudomucin.

The material for the final example of foreign body granulomatosis in this group was from a young married woman, 26 years of age, who was operated upon for what appeared to be an attack of acute appendicitis, but which proved to be an appendix of the interval type. At operation the mesoappendix, broad ligaments and pelvic peritoneum were covered with small clear nodules containing a non-fatty gelatinous material of undetermined source. Sections showed typical foreign body granulomas with numerous large giant cells. The gelatinous material contained many concretions suggesting starch granules. The possibility of a gelatinous contraceptive as the source of this material was suggested. This seemed possible, as Dr. Mortimer Cohen recently had a case * of multiple granulomas

* This case was presented at a meeting of the Pittsburgh Clinical-Pathological Society.

of the tubes following lipiodol injections for roentgen-ray examination. The use of such substances was denied by the patient and the lesions remained unexplained. However, the possibility of gelatinous contraceptives as a source of granulomas seems practicable.

(*d*) *Foreign Body Granulomas about Oils and Free Fats:* There are at least three kinds of oily substances that may give rise to foreign body granulomas in the peritoneal cavity. These include extraneous oils and fatty compounds introduced during operation, sebaceous material from ruptured dermoid cysts, and the later stages of fat necrosis associated with diseases of the pancreas.

At one time it was a fairly common practice to introduce oily substances into the abdomen to prevent peritonitis and adhesions. Wilkie [12] in 1910, at which time this procedure was being widely considered, published an article discussing the relative merits of olive oil, vaseline, mucilage, gum arabic, paraffin, lanolin, gelatin-agar and fibrolysin for the prevention of adhesions and peritonitis. He recommended vaseline smeared over the intestines as the best of the group because it did not break down into irritating fatty acids and it retarded the absorption of bacteria. Such practices were soon found to produce, rather than prevent, adhesions and were abandoned.

A case report and review of several cases of ruptured dermoid cysts by Herzog [13] throws light on the fate of free fats in the peritoneal sac. Herzog's case was that of a 68 year old woman who had had a large dermoid cyst ruptured in an accident fifteen days before death. At autopsy the peritoneum was covered with nodules and there were also 800 gm. of free fat, sebaceous material and hair in the cavity. Some of the fat appeared to have separated into free droplets and fatty acids and to have become fixed to the walls by fibrin. This material was quickly overgrown by the mesothelium and became organized through the ingrowth of fibroblasts and the formation of foreign body giant cells and capillaries. Some of the granulomas were found deeply embedded in the subperitoneal tissues. Among the other cases cited by Herzog was one of Fränkel's, in which the squamous epithelium of a ruptured dermoid cyst coated the peritoneum with an epithelial layer resembling that of the skin.

The appearance of early fat necrosis in the peritoneum and omentum is now widely recognized. It does not seem to be well understood that foci of fat necrosis sometimes persist for weeks and give

rise to typical granulomas difficult to differentiate microscopically from those formed about extraneous fats. In his experimental studies Wells [14] followed the development of fat necrosis after the injection of pancreatic juice. He concluded that both trypsin and lipase were necessary, trypsin to produce the necrosis and lipase to act in breaking down the fats. He further determined that bile salts may produce necrosis but do not digest fats. Free fat droplets and soaps so liberated were not toxic, though they often took up calcium and became insoluble. Experimental lesions disappeared after eleven days. Distant lesions seemed to be due to emboli of pancreatic cells. Rewbridge [15] found that injected bile salts produced a special form of fat necrosis in normal dogs, but caused hemorrhages without necrosis in depancreatized dogs. He concluded that after the escape of bile salts, fat necrosis may occur independently of pancreatic disease though not independently of pancreatic juice. The observation is of especial interest in explaining several of our cases of fat necrosis associated with long-standing gall-bladder disease.

In our series there were eight cases of foreign body granulomatosis of the peritoneum due to fatty substances. Three were interpreted from the findings as being due to extraneous oily substances.

In the first of these cases the patient had had two previous operations, one of which was a gastro-enterostomy performed several years before. On opening the abdomen there were extensive adhesions between the coils of intestines, with numerous cyst-like bodies attached closely or by short pedicles over the sigmoid and uterus. There were numerous cyst-like inclusions in the omentum filled with an oily substance soluble in alcohol and chloroform. A clinical diagnosis of granulomatosis about oil used at a previous operation was made.

Microscopically the cyst-like bodies resembled normal fat with extensive infiltration between the globules. Closer observation showed them to be made up of closely packed, clear, globular spaces surrounded by giant cells. Between the globules there was a fibroblastic granulation tissue infiltrated with mononuclears, lymphocytes and eosinophiles, particularly marked about the vessels. There were large areas packed with masses of multinucleated cells containing clefts and irregular cysts.

The second case classed as due to extraneous fats was discovered during the repair of an incisional hernia. The patient had been oper-

ated upon three times between 1912 and 1915 for a supposed ulcer of the duodenum. The surgeon stated that there were so many adhesions he could not tell what had been done, though he assumed that oil had been used at some previous time.

The third case occurred about a walled-off area in the ileocecal fossa following the repeated packing of a sinus with bismuth paste. Both the tract and the adhesions about the cecum were filled with dark nodules so that a secondary melanotic sarcoma was suspected.

Microscopic examination showed globular spaces, for the most part empty, though a few were lined with black pigment and surrounded by large, multinucleated giant cells. Other types of exudative cells were almost entirely absent in this lesion.

Of the five cases associated with pancreatic and gall-bladder disease one is of interest because the date of onset was known definitely. This patient, aged 7 years, was struck by an automobile and suffered several fractures, as well as internal injuries. The abdomen began a gradual and continued enlargement and an exploratory operation was performed forty days after the accident. An encysted peritonitis containing 500 cc. of fluid was found in the lesser peritoneal sac. The peritoneum and omentum appeared inflamed and were studded with small, discrete, yellowish white tubercles. A diagnosis of tuberculous peritonitis was made.

Microscopic examination proved the nodules to be foci of fat necrosis which were practically all encapsulated and in which there were a fair number of giant cells. The patient recovered.

In another case in this group there was a history of a recurrent attack of abdominal pain preceding death. At autopsy the findings included acute hemorrhagic pancreatitis, fresh foci of fat necrosis in the peritoneum and completely organized granulomatous nodules about areas of fat necrosis. The latter were probably accounted for by the earlier attack.

Of the three remaining cases of encapsulated areas of fat necrosis, in one case this condition was associated with carcinoma of the pancreas, in the second with repeated attacks of cholecystitis, and in the third with cholelithiasis. In this last case the surgeon found the head of the pancreas enlarged and unusually firm. In the latter two cases free fat droplets, organization and giant cell formation were present. Actual necrosis was absent (Fig. 1).

(*e*) *Foreign Body Granulomas about Extruded Stomach and Intestinal*

Contents: When an ulcer of the stomach ruptures and the stomach contents escape into the peritoneal cavity a fatal peritonitis generally ensues. Occasionally enough of the material is removed at an emergency operation to permit recovery. Again the peritoneum is able to take care of the bacterial contamination, and food particles are left to produce foreign body reactions. Occasionally there is a case reported where vegetable matter has been found in the peritoneum, without a history of previous operation or a definite history of an acute abdominal attack. Shennan [16] reported such a case and identified the vegetable particles as encapsulated oat seeds. In newly born infants foreign body granulomas have been described about extruded meconium (Boikan [17]). We have not found any instances of meconium peritonitis of this type, though we have had four cases of foreign body granulomas about vegetable material in our series. The first of these was mentioned in the introductory paragraph. A brief summary of this case follows.

The patient was first admitted with a duodenal perforation of about four hours duration. At operation a large calloused ulcer was found. The abdomen contained considerable fluid and stomach contents, in which the coverings of peas were recognized. The perforation was closed and a gastro-enterostomy performed. The patient recovered and remained well for six months when he began to have discomfort in the region of the stomach and retention of gastric contents. Ten months after the first operation he was readmitted to the hospital. At operation the omentum was found to be rolled up and adherent to the colon and to the abdominal scar. In the right upper quadrant there was a mass of adhesions which were broken with difficulty. All through the omentum and adherent to the colon were small, white, lymph node-like nodules varying from a pin-point to 0.5 cm. in diameter. A clinical diagnosis of tuberculous peritonitis was made and a piece of tissue sent to the laboratory for a frozen section. This was found to be a foreign body granuloma formed about vegetable matter. Further study revealed occasional areas that contained bits of striped muscle. Figure 2 shows a nodule from this patient.

Following this case a series of animals was injected with stomach contents. When untreated contents were used the animals died of peritonitis. When cooked sterile contents were used the lesions found were reproductions of the granulomas seen in the case men-

tioned above. Cooked muscle tissue injected into guinea pigs intraperitoneally was found to persist still undigested and without loss of striae when the animals were killed ten weeks later.

In the second case the patient was an Italian laborer, 52 years of age, who had been injured in a fall some months previous to admission. He was admitted to the hospital for the repair of inguinal and ventral hernias. He stated that he had not been sick a day in his life. When the abdomen was opened the peritoneum, omentum and intestinal serosa were thickly studded with nodules resembling tubercles. A diagnosis of tuberculous peritonitis was made and specimens sent to the laboratory. They were much like the granulomas in the first case, though their origin was unknown.

The third case was discovered at operation when a loop of bowel which had ruptured six weeks before and which had been pulled up and sutured outside the abdomen was being returned to the peritoneal cavity. Again a diagnosis of tuberculous peritonitis was erroneously made.

The fourth case followed the rupture of a stomach ulcer nine months earlier. At autopsy this proved to be a carcinoma of the stomach. The peritoneum and omentum were studded with nodules, which on microscopic examination proved to be secondary growths. In two of them, however, there were found encapsulated vegetable remains.

(*f*) *Foreign Body Granulomas about Sutures:* There have been a great many small localized granulomas about sutures in our material, but they have been incidental findings and have caused no difficulty in diagnosis.

Summary

A series of nearly thirty cases of nodular lesions of the peritoneum, which were of interest from the standpoint of surgical diagnosis, have been collected from our material and an outline has been prepared to facilitate the more rapid classification of such lesions.

The writer begs to express his most sincere thanks to the various members of the surgical and gynecological staffs of the Allegheny General Hospital for permission to cite their cases.

REFERENCES

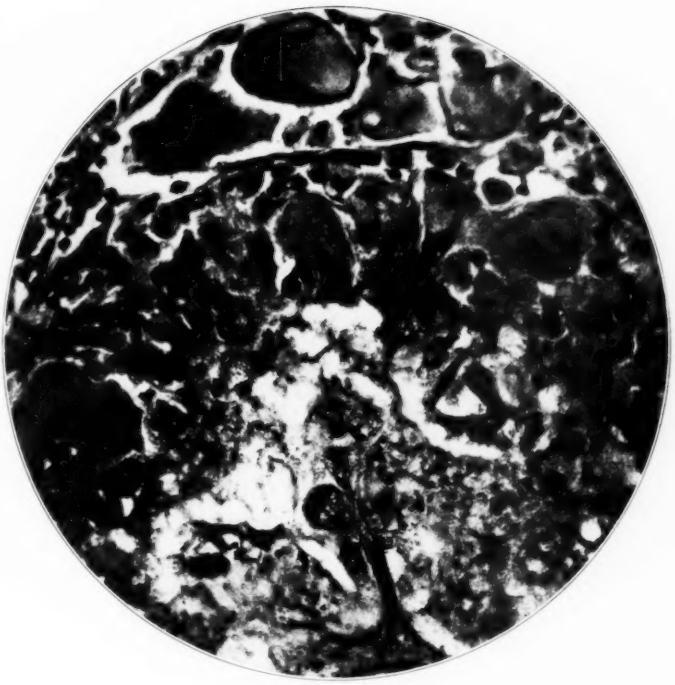
1. Kaufmann, E. Pathology for Students and Practitioners. Translated by S. P. Reimann. P. Blakiston's Son & Co., Philadelphia, 1929, **2**, 870–881.
2. Hertzler, Arthur E. The Peritoneum. C. V. Mosby Company, St. Louis, Mo., 1919, **2**, 713.
3. Reimann, H. A., and Rose, W. J. The similarity of pseudotuberculosis and tularemia. *Arch. Path.*, 1931, **11**, 584–588.
4. Gunn, H., and Howard, N. J. Amebic granulomas of the large bowel. *J. A. M. A.*, 1931, **97**, 166–170.
5. Goodale, R. H., and Krischner, H. Oxyuris vermicularis in the peritoneum. *Arch. Path.*, 1930, **9**, 631–634.
6. Del Campo, J. C. Hydatid peritoneal pseudotuberculosis. *Ann. Fac. de med. Univer. de Montevideo*, 1927, **12**, 702. Abstr. in *Internat. Abstr. Surg.*, 1928, **47**, 310.
7. Hutchison, Harry S. The pathology of bilharziasis. *Am. J. Path.*, 1928, **4**, 1–16.
8. Sampson, J. A. Intestinal adenomas of endometrial type. *Arch. Surg.*, 1922, **5**, 217–280.
9. von Gierke, E. Storungen des Stoffwechsels. Pathologische Anatomie, Aschoff, L. G. Fischer, Jena, 1928, Ed. 7, 440.
10. Dieker, W. Ueber das Pseudomyxoma peritonei. *Virchows Arch. f. path. Anat.*, 1931, **282**, 761–771.
11. Frank, R. T. Gynecological and Obstetrical Pathology. D. Appleton and Company, New York and London, 1931, Ed. 2, 374.
12. Wilkie, D. P. D. The use of oil in abdominal surgery. *Surg. Gynec. Obst.*, 1910, **10**, 126–132.
13. Herzog, Georg. Ueber die Einheilungsvorgänge am Peritoneum nach Ruptur einer Dermoidcyste des Ovariums; 14 Jahre alte Ausschaltung einer Darmschlinge. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1912, **53**, 371–428.
14. Wells, H. Gideon. Chemical Pathology. W. B. Saunders Company, Philadelphia, 1925, Ed. 5, 431.
15. Rewbridge, A. G. Fat necrosis in bile peritonitis. *Arch. Path.*, 1931, **12**, 70–76.
16. Shennan, T. Foreign-body tubercles on serous coat of stomach, caused by escape of particles of oat-seed. *J. Path. & Bact.*, 1923, **26**, 82–84.
17. Boikan, W. S. Meconium peritonitis. *Arch. Path.*, 1932, **14**, 50–59.

DESCRIPTION OF PLATE

PLATE 119

FIG. 1. Foreign body granuloma of the mesentery due to the organization of fat necrosis in a case of chronic cholecystitis and cholelithiasis without rupture. × 150.

FIG. 2. Foreign body granuloma of the peritoneum due to the extrusion of vegetable material through a ruptured duodenal ulcer. This lesion was of ten months duration. The condition was diagnosed tuberculosis on inspection of the peritoneum at the second operation. × 30.



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GROWTH INHIBITOR IN KIDNEY DESICCATES *

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As early as 1913 Carrel [1] determined that fibroblasts and epithelial cells multiply indefinitely in a medium of plasma and embryonic juice. In 1926 Baker and Carrel [2] found that the agent responsible for the multiplication was contained in the protein fraction of the embryonic juice, and later the same year proved that proteoses prepared from many different proteins stimulated the proliferation of fibroblasts and other cells.[3] Their explanation of the stimulating effect of embryonic juice was that the enzymes contained in it hydrolyzed the protein to proteose. Peptones, peptids and amino acids resulting from further hydrolysis contributed to the nutrition of the fibroblasts.[4] These experiments leave little doubt as to the power of certain primary protein derivatives to excite karyokinetic activity in cells removed from the body and placed *in vitro* under conditions favorable for growth. Within the body of an animal the cell is in an environment made so complex by chemical, nutritional and nervous influences that a test of the action of substances on mitotic activity is not easy to make. Carrel [5] found that the application of dog subcutaneous tissue to skin wounds of dogs delayed cicatrization. The author [6] observed some stimulating effect of macerated epidermis, especially that of fetal origin, on the regeneration of epidermis in cutaneous wounds. Dvorak and Byram [7] applied various macerated tissues such as liver, kidney and spleen to wounds of the skin, but found no striking acceleration of healing.

Physiological processes on analysis often are found to consist of two components which operate in opposition to each other. Some biologists have expressed the view that the mechanism of tissue growth consists of a balance and imbalance of inhibitory factors and formative stimuli. Evidence of the presence *in vitro* of definite growth stimuli is satisfactory. Proof of their existence within the animal is less certain. When inhibition within the body is considered the situation is very different. Several inhibitors are known as defi-

* Received for publication April 3, 1933.

nite substances with specific properties. Up to now most but not all of the investigations along this line have been with organs producing hormones. Halsted [8] successfully transplanted parathyroid only when one-half or more of the parathyroid tissue had been removed. Loeb [9] found active thyroid regeneration only after the operative removal of about three-fourths of the thyroid gland and, of even greater significance, he observed an inhibition of regeneration in partially thyroidectomized animals that were placed on thyroid feeding.

We began our study of the operation of inhibitors of cell proliferation with observations on the pancreatic islets of suckling rats injected with insulin.[10] The results were decisive. Up to 20 days of age mitoses were abundant in the pancreatic islets of rats. Iletin injected intraperitoneally reduced the mitotic rate from one-third to one-tenth that of the normal, with no adverse action on the general nutrition of the animal. In fact growth, as shown by the total body weight, was in several instances accelerated. The effect of parathyroid hormone on the proliferative activity of the parathyroid glands was determined.[11] In order to estimate the hormonic effect by a determination of the hypercalcemia we had to employ half-grown rats, and the rate of parathyroid proliferation in these was such that an accurate enumeration of all the mitoses in the entire glands was necessary. Formalin-fixed tissue proved to be entirely unsatisfactory for this purpose. A particular chrome-formol fixation gave a clear definition of the karyokinetic figures. Maximum, minimum and average rates of proliferation were then established in a series of normal rats of the same age as those used in the hormone injections. The injections of two different preparations of parathyroid hormone reduced the proliferative rate to a fraction of the normal. This reduction was accomplished not only by the hypercalcemic dosage but by relatively small doses which produced no demonstrable change in serum calcium. The reduction in mitotic rate was found to be a more delicate test for hormonic activity than either hypercalcemia or the occurrence of toxic lesions in parenchymal organs. We made several quite instructive tests with an inactivated hormone prepared by Tweedy and Torigoe.[12] By a chemical change in the hormone its hypercalcemic action is removed (inactivation), but the process is reversible and its property of producing a hypercalcemia may be restored (reactivation). The inacti-

vated hormone, perfectly inert as far as blood calcium and toxicity are concerned, was equally as effective as the active hormone in its inhibiting action on the mitotic rate of the parathyroid gland.

Microchemical tests of the effect of epinephrin on the adrenal medulla were made by Ogata and Ogata [13] who, using toluidin blue as a histological reagent, observed that the depth of green in the medullary cells was proportional to the amount of epinephrin or preëpinephrin substance present. They found only a trace of green after adrenalin administration. Samson [14] confirmed these findings by demonstrating reduced amounts of epinephrin content in the medullary cells in dogs receiving intravenous epinephrin. Further confirmation of a diminished content of secretion within the gland following dosage with adrenalin was obtained by Cramer,[15] who observed that the cells of the adrenal medulla lose their chromaffin staining after injections of epinephrin. He concluded from this that the adrenal gland has the power to control its functional activity in such a way as to prevent the gland from stimulating itself to complete exhaustion. Employing suckling rats we established the normal proliferative rate of the adrenal medulla for the ages used in the experiments.[16] The epinephrin was injected intraperitoneally, usually into litter-mates of the control animals. The mitotic rate was reduced by the injections to a fraction of the normal, one-half to one-twenty-fifth. Excess of epinephrin therefore not only inhibits the function of the individual cells but prevents the formation of new cells.

In the work that we shall now describe we studied the effects of extracts of an organ, the kidney, not known to produce an internal secretion. In the previous experiments hormones were available for the tests. In the case of the kidney hormones were neither available nor known to exist. However, in our preceding work a method had been established for determining the effect of injected substances on the proliferative activity of the tissues of the recipient animal. In the experiments that follow extracts were prepared from the rat kidney and the effect on mitotic rate was determined in the kidney of rats injected intraperitoneally with the extracts. In all experiments a single intraperitoneal injection of the quantity indicated was made and the animal killed twenty-four hours later. At first simple aqueous extracts were prepared and tested. Then less simple extractions and fractionations were carried out. A uniform method

of testing the extracts was used in all experiments.[11] Rats unweaned, about 25 days of age, were chosen. Litter-mates were used as controls, and these with the rats used for testing a particular extract or eluate, are given consecutive numbers in the tables to indicate each group. Members of the different litters showed a wide range in mitotic rate. The rats were kept on a standard complete diet and no change in their environment was made at the time of the experiment.

All animals were killed and the right kidney fixed immediately in formalin for paraffin embedding and for sectioning. A transverse block 3 mm. thick was cut from the kidney midway between the poles. Ribbons of sections cut at 8 microns were mounted from the center of this block and stained. The mitoses present in the renal tubules in three to five sections were enumerated.

The extracts were prepared from fresh kidney and from desiccated kidney of full grown rats. The etherized animals were bled by slitting the great vessels of the neck. With aseptic precautions the fibrous capsules were stripped from the kidneys and the capsules, together with the renal vessels and all peripelvic fat and fibrous tissues, were removed. The kidneys were then ground thoroughly in a mortar and the ground mass spread on a glass plate for desiccation, which was accomplished by playing an electric fan on the plate for about two hours. By spreading in a very thin layer the pulp was reduced to a hard scale within five minutes. The material was scraped from the glass with the aid of a safety razor blade and ground to a fine powder in a mortar. It was then placed in a bottle over phosphorous pentoxid and the air exhausted. Various extracts were prepared from the desiccated kidneys.

Simple Aqueous Extracts of Fresh Macerated Kidney and of Desiccated Kidney (Table I)

Extract 1: To 3.2 gm. of fresh kidney pulp 16 cc. of distilled water were added and the supernatant fluid collected by centrifugation. Large doses yielded inconclusive results (Rats 1–4).

Extract 2: To 1.3 gm. kidney desiccate 13 cc. of distilled water were added. The mixture was centrifuged and the supernatant fluid collected. This extract reduced the mitotic rate to a fraction of that of the controls (Rats 10–12).

Extract 3: To 1.4 gm. kidney desiccate 28 cc. of distilled water were added and the supernatant fluid collected as in the other extracts. In this experiment the number of mitoses was reduced (Rats 20–22).

Extract 4: This extract was made by filtration of the supernatant fluid from kidney desiccate through filter paper several times. The supernatant fluid was prepared as in Extract 2. Although coarse particles in suspension were removed by the filtration the inhibitor was not materially lessened (Rats 30–35).

TABLE I

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	6 cc. Extract 1	26	3.0
2	6 cc. Extract 1	26	1.0
3	Control	26	8.2
4	Control	26	3.0
10	4 cc. Extract 2	27	0.2
11	Control	27	9.2
12	Control	27	7.2
20	4 cc. Extract 3	25	5.8
21	5 cc. Extract 3	25	4.0
22	Control	25	17.2
30	0.5 cc. Extract 4	25	4.2
31	1 cc. Extract 4	25	8.4
32	2 cc. Extract 4	25	2.4
33	4 cc. Extract 4	25	2.8
34	Control	25	23.0
35	Control	25	16.6

Filtrates of Simple Aqueous Extracts of Desiccated Kidney (Table II)

Our next step was an attempt to remove by filtration all cell particles held in suspension. The extracts of the filtrates were made

Table II

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	4 cc. Extract 1	23	11.6
2	Control	23	37.8
3	Control	23	44.0
4	Control	23	28.8
10	2.7 cc. Extract 1	25	22.2
11	2.7 cc. Extract 1	25	22.6
12	Control	25	36.0
20	2.5 cc. Extract 1	24	15.2
21	Control	25	15.4
30	4 cc. Extract 2	28	0.8
31	Control	28	0.7
40	5 cc. Extract 3	25	33.6
41	Control	25	24.4
50	4.25 cc. Extract 3	24	12.5
51	Control	24	12.8

as given in Table I, except that the ratio of water to desiccate was somewhat increased.

Extract 1: To 1 gm. of kidney desiccate 20 cc. of distilled water were added and the supernatant fluid collected as before. This extract was passed through a Mandler bougie. There was a reduction in mitotic rate of only 17 per cent. In a second extract there was practically no reduction.

Extract 2: This was prepared as in Extract 1, except that the fluid was passed through a Berkefeld N filter. The inhibiting principle was removed.

Extract 3: An attempt, which was unsuccessful, was made to adsorb the inhibiting agent contained in Extract 1 (Table I) by aluminum hydroxid. The aluminum hydroxid designated by Wilstätter and Kraut [17] as Type C was employed. Murphy and coworkers [18] used this preparation to adsorb the active principle of Rous Chicken Tumor I. Later Murphy and Sturm [19] determined

TABLE III

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	2 cc. Extract 1	24	13.0
2	Control	24	59.5
3	Control	24	40.5
10	2 cc. Extract 2	26	3.3
11	0.3 cc. Extract 3	26	4.6
12	0.125 cc. Extract 3	26	3.6
13	Control	26	3.0

that an inhibiting substance was adsorbed along with the active principle. We recognized in all these experiments that we might be dealing with a combination of stimulant and inhibitor.

Acid-Aqueous Extracts of Desiccated Kidney (Table III)

Extract 1: To 2.3 gm. of desiccated kidney 46 cc. of N/1 HCl were added. The mixture was placed in a boiling water bath for one hour. The supernatant fluid was obtained and made just alkaline to litmus with N/1 NaOH. The precipitate obtained by centrifugation was taken up with 3 cc. of distilled water and the solution made neutral to litmus. The total volume was 4 cc.

Extract 2: Made by same procedure as that used in the preparation of Extract 1.

Extract 3: Supernatant liquid above the isoelectric precipitate of Extract 2.

The extractions were made with relatively strong acid and at a high temperature. Both the isoelectric precipitate and the supernatant liquid were tested. In consideration of the large quantity of kidney represented in the dosage the first experiments with the precipitate gave little or no evidence of inhibition (Rats 1–3). A second test of another preparation was negative (Rats 10 and 13). The liquid above the precipitate was toxic in large doses. Sublethal doses did not inhibit (Rats 11–13). A tentative conclusion at this time assumed that the inhibitor was destroyed by the acid, high temperature, or the two in combination.

TABLE IV

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	4 cc. Extract 1	24	10.0
2	3 cc. Extract 1	24	11.0
3	Control	24	18.3
4	Control	24	11.0

ACID-AQUEOUS EXTRACTS OF FRESH MACERATED KIDNEY (TABLE IV)

Extract 1: To 9 gm. of fresh kidney 18 cc. of 3 per cent HCl were added. The mixture was placed at 70° C for twenty minutes and then filtered through muslin. After neutralization to Congo red with N/1 NaOH an equal volume of acetone was added and the mixture placed in the ice-box overnight. The clear supernatant liquid obtained by centrifugation was reduced to a small volume by fanning and then made up to 21 cc. with water.

The method differed from the preceding one recorded in Table III in several particulars. The extraction was made at a somewhat lower temperature, and fresh minced kidney was extracted. In the quantities used the extract was without effect on the mitotic rate of the renal epithelium.

Acid-Alcohol Extracts of Desiccated Kidney (Table V)

Extract 1: To 0.7 gm. desiccated kidney there were added 14 cc. of 60 per cent alcohol made 0.2 per cent acid with HCl. The mixture was kept at room temperature for one hour. The supernatant fluid was obtained by centrifugation. After fanning off the alcohol water was added and the liquid neutralized with N/1 NaOH. Water was then added to make the total volume 5 cc.

Extract 2: Preparation by the method used for making Extract 1. In Extract 1 the strength of acid was reduced and a low tempera-

Table V

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
		days	
1	1 cc. Extract 1	24	7.6
2	4 cc. Extract 1	24	9.0
3	Control	24	42.0
4	Control	24	62.0
10	3 cc. Extract 2	24	2.6
11	2 cc. Extract 2	24	2.0
12	Control	24	20.0
13	Control	24	8.3

ture employed. Rats 1–4 inclusive withstood a large dose and the inhibition was pronounced. Another preparation was made and the experiment repeated (Rats 10–13). Again the inhibitory action was decisive.

Acid-Alcohol Extracts of Fresh Kidney (Table VI)

Extract 1: To 15 gm. fresh macerated kidney 45 cc. of 95 per cent alcohol, 0.36 cc. 95 per cent H_2SO_4 and 15 cc. of water were added and the mixture heated at 40° C for two hours, and at 20°–25° C for three hours. The extract obtained by filtration through muslin

was made neutral to litmus by the addition of N/1 NaOH and the supernatant liquid obtained by centrifugation. After removal of the alcohol by fanning, sufficient water was added to make the total volume 30 cc.

Extract 2: After bringing the pH of Extract 1 to pH 4.8 the concentration of alcohol was made up to 75 per cent. After standing

TABLE VI

No. of rat	Dosage	Age at autopsy	No. of mitoses per section
1	0.1 cc. Extract 1	24	4.0
2	0.16 cc. Extract 1	24	13.0
3	0.2 cc. Extract 1	24	7.3
4	0.2 cc. Extract 1	24	2.3
5	Control	24	45.0
6	Control	24	15.6
10	0.9 cc. Extract 2	25	1.0
11	Control	25	20.0
12	Control	25	18.3
20	1 cc. Extract 2	24	5.3
21	1 cc. Extract 2	24	8.6
22	Control	24	45.0
23	Control	24	15.6

forty hours in the ice-box the precipitate was separated by centrifugation. The supernatant fluid was reduced to a small volume by fanning and made up to original volume with water.

In Extract 1 a large flocculent isoelectric precipitate was removed and the supernatant liquid proved to be inhibitory (Rats 1–4). This liquid was further purified by alcoholic precipitation which removed sulphate and protein. Its inhibitory potency was not affected by the purification (Rats 10–12, and Rats 20–23).

Discussion and Summary

It is well known that certain organs elaborate chemical regulators or hormones which govern growth and metabolism. It has been demonstrated that several of these inhibit karyokinesis of the cells that produce them. This self-regulatory function of the hormones is not known to depend on their metabolic activity. In the case of the parathyroid the hormone made inactive, as far as calcium metabolism is concerned, still inhibits proliferation of the parathyroid cells. The experiments recorded in this paper describe methods for the extraction from rat kidney of an inhibitor of renal tubule mitosis. It is not known that this inhibiting agent exercises any control over growth of the body as a whole, or that it plays any rôle in metabolism.

Simple aqueous extracts made from the fresh macerated kidney had little influence on the proliferation of renal epithelium. Simple aqueous extracts of desiccated kidney, however, were inhibitory when sufficiently large doses were injected. A more satisfactory potency was obtained by means of acid-alcohol extraction of either fresh or desiccated kidney. The successful extracts were made with dilute acid, 60 per cent alcohol, and at temperatures of 40° C or less. Those acid-aqueous extracts that were tested were without effect on the renal epithelium. They were prepared at 70° C or more and the strength of acid was relatively strong. Further attempts are being made to purify and concentrate the extract.

REFERENCES

1. Carrel, A. Artificial activation of the growth *in vitro* of connective tissue. *J. Exper. Med.*, 1913, **17**, 14–19.
2. Baker, L. E., and Carrel, A. Action on fibroblasts of the protein fraction of embryonic tissue extract. *J. Exper. Med.*, 1926, **44**, 387–395.
3. Carrel, A., and Baker, L. E. The chemical nature of substances required for cell multiplication. *J. Exper. Med.*, 1926, **44**, 503–521.
4. Baker, L. E., and Carrel, A. Nitrogen metabolism of normal and sarcomatous fibroblasts in pure cultures. *J. Exper. Med.*, 1928, **48**, 533–547.
5. Carrel, A. The process of wound healing. *Proc. Inst. Med. Chicago*, 1930, **8**, 62–66.
6. McJunkin, F. A., and Matsui, T. Effect of homologous macerated skin on the regeneration of epidermis. *Arch. Path.*, 1931, **12**, 794–801.

7. Dvorak, H. J., and Byram, J. W. Effect of addition of macerated tissues on healing of granulating wounds. *Proc. Soc. Exper. Biol. & Med.*, 1930, **27**, 967–969.

8. Halsted, W. S. Auto- and isotransplantation, in dogs, of the parathyroid glandules. *J. Exper. Med.*, 1909, **11**, 175–199.

9. Loeb, L. Studies on compensatory hypertrophy of the thyroid gland. *J. Med. Res.*, 1919–20, **41**, 481–494.

10. McJunkin, F. A., and Roberts, B. D. Effect of excessive insulin on the pancreatic islets of young rats. *Proc. Soc. Exper. Biol. & Med.*, 1932, **29**, 893.

11. McJunkin, F. A., Tweedy, W. R., and Brenhaus, H. C. The parathyroid hormone. Its regulatory action on the parathyroid glands and toxic effect on the tissues of the rat. *Arch. Path.*, 1932, **14**, 649–659.

12. Tweedy, W. R., and Torigoe, M. Chemical studies on a parathyroid hormone. *J. Biol. Chem.*, 1932, **99**, 155–164.

13. Ogata, T., and Ogata, A. Henle's reaction of the chromaffin cells in the adrenals, and the microscopic test for adrenalin. *J. Exper. Med.*, 1917, **25**, 807–817.

14. Samson, P. C. Tissue changes following continuous intravenous injection of epinephrine hydrochloride into dogs. *Arch. Path.*, 1932, **13**, 745–755.

15. Cramer, W. Self-control and inhibition in the adrenal gland. *Brit. J. Exper. Path.*, 1926, **7**, 88–93.

16. McJunkin, F. A., Rall, R. R., and Singer, P. L. Effect of adrenaline chloride on the proliferative activity of cells of the adrenal medulla. *Endocrinology*, 1932, **16**, 635–638.

17. Wilstätter, R., and Kraut, H. Zur Kenntnis der Tonerdehydrate. *Ber. d. deutsch. chem. Gesellsch.*, 1923, **56**, 149–162.

18. Murphy, J. B., Sturm, E., Claude, A., and Helmer, O. M. Properties of the causative agent of a chicken tumor. *J. Exper. Med.*, 1932, **56**, 91–106.

19. Murphy, J. B., and Sturm, E. Properties of the causative agent of a chicken tumor. *J. Exper. Med.*, 1932, **56**, 107–116.

THE REACTION TO FINE AND MEDIUM SIZED QUARTZ AND ALUMINUM OXIDE PARTICLES. SILICOTIC CIRRHOSIS OF THE LIVER.*

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The experiments to be described were performed as part of a general program for study of the reaction of the body to various inorganic dusts. Previous investigation had demonstrated that it is possible to reproduce the typical nodular lesions of silicosis in guinea pigs and rabbits by exposing them for 8 hours daily over a period of 1 or more years to the inhalation of fine silica dust.[1] These observations failed to indicate the nature of the injury produced by silica and they did not define the quantity and size of the particles necessary to provoke reaction. The present paper deals with preliminary attempts to evaluate the importance of the factor of size. It also throws further light upon the nature of the injury.

It is well established that the reaction to silica differs from that to any other known type of dust and it has been hypothecated that the biological activity of this substance is due to its solution in the slightly alkaline fluids of the body. This hypothesis is based upon the clinical observations of Collis [2] and upon certain experiments of Gye and Purdy.[3] The latter investigators showed that a colloidal suspension of silica is toxic. Intravenous injection of large doses produces almost immediate death; repeated smaller injections injure various cells and later stimulate proliferation of connective tissue. Subsequent writers have erroneously assumed that the silica sol used by Gye and Purdy was a chemical solution, whereas it was actually a colloidal suspension of exceedingly fine particles. More recently Heffernan [4] has suggested that silica is active when freshly fractured because of its molecular structure, which presents numerous unsatisfied oxygen atoms to interact with tissue elements. This hypothesis would demand that the reaction produced by a given quantity of silica be proportional to the size of the particles, since a larger num-

* Received for publication April 13, 1933.

ber of unsatisfied oxygen atoms would be exposed with successive increases in subdivision. Regardless of the mechanism involved all available evidence points to a chemical rather than a physical type of injury by silica.

As originally planned, these experiments were designed to demonstrate the relation of particle size to the amount of reaction that would be produced by a measured quantity of silica. If such reaction were to take place at the surface of the particles it should be proportional to the amount of surface exposed to the body fluids. Since a known quantity of material must be introduced into the body for such a study intravenous injection was substituted for inhalation.

Two series of 8 rabbits each were injected with 1.3 gm. of silica particles. For the first series the particles were approximately the size of leukocytes, being 6 to 12 microns in maximum diameter; for the second, they were about one-tenth as large (1 to 3 microns). To control the action of the silica a third series of 8 rabbits was injected with 1.2 gm. of aluminum oxide particles of the same size as those of the smaller silica fraction (1 to 3 microns). This substance was selected because its chemical properties simulate those of silica more closely than those of any other known substance. The alumina employed was an electric furnace product known as "aloxite." The size of all particles was carefully graded by a process of fractional sedimentation which has been described elsewhere.[5] Two per cent suspensions by weight were made in physiological salt solution and sterilized in the autoclave. It was originally intended to inject each animal with 10 cc. each week of the experiment, but it soon became apparent that this program could not be followed as the fine quartz particles provoked so much reaction in the ears that long periods of rest between injections were necessitated. As a consequence from 1 to 4 months elapsed before the full 1.3 gm., often in doses as small as 3 cc., could be administered. Seven of the 24 animals died of embolism during the course of treatment and 2 more in the group receiving the finer quartz particles died subsequently of silicosis. One animal died during the first injection and showed coccidiosis, but the others were entirely free from any trace of this infection. The remaining members of the three groups were killed from 2 to nearly 3 years after completing the injections.

Experimental Results

Fine Silica Particles (1 to 3 Microns)

This material is irritating and it tends to produce thrombosis in the ear veins, followed by accumulations of the dust in the surrounding tissues.

Ear: In this location the dust provokes a localized necrosis with an acute exudation of polymorphonuclear leukocytes and a small amount of fibrin. The masses of dust have persisted with little decrease in their size throughout the entire period of observation (1067 days). They become surrounded by a zone of mononuclear leukocytes and this in turn is gradually replaced by connective tissue which is at first cellular and later hyaline in character.

Lungs: The particles of this dust fraction are so fine that only a few of the larger ones are retained in the capillaries of the lungs. They are gradually phagocytosed and transported into the perivascular lymph spaces and thence into the pulmonary lymphoid tissues. A small number ultimately reach the tracheobronchial lymph nodes. The amount of silica in any one spot is so small that very little fibrosis results. Of 2 animals killed at the end of the experiment, 1 shows only phagocytes containing dust particles in the intra- and extrapulmonary lymphoid tissues, and the other shows a limited amount of loose, non-cellular, fibrous tissue in the walls of the air spaces adjacent to the lymphoid tissues.

Spleen: The spleen seems to have filtered out and retained most of the silica particles of intermediate size in this fraction. They are found in large, swollen phagocytes with from one to four nuclei. The number of such particles seen in a section of any one cell cut at 6 microns rarely exceeds eight to ten. There are also smaller particles, only visible with polarized light, which are collected in great numbers by exceedingly large giant cells. A section of such a cell may exhibit as many as thirty or more nuclei massed in its center or arranged in ring formation about its periphery. They are indistinguishable from the Langhans giant cell of tuberculosis.

Three months after completing the injections compact masses of such cells are found in dilated portions of the sinuses. In another 6 months these lesions have increased to nearly 1 mm. in diameter and they often extend through the walls of the sinuses into the septa

and follicles. Blood vessels in the area involved are not destroyed but appear normal at this time. After a year or more the nodules of phagocytes have been replaced by considerably larger nodules of cellular connective tissue exhibiting numerous mitotic figures. They have clearly defined borders and are covered with a thin layer of flat cells, probably sinus endothelium. At their centers the tissue becomes progressively fibrous in character and in the last animal killed a small amount of specific silicotic hyalin has begun to appear. This change is attended by no apparent alteration in the blood vessels.

Liver: The sinusoids of the liver have arrested and retained the major part of the finest particles in this fraction and as these were in great excess, the amount of reaction in this organ is much more pronounced than in any other. No animal was killed sufficiently early to observe the immediate cellular response in this location, but the experiences with larger particles, to be discussed below, would indicate that they are probably phagocytosed by Kupffer cells, which become detached and migrate with their ingested material toward the portal areas. In the first animal killed at 84 days there are numerous multinucleated, dust-containing cells, which are probably still within the sinusoids of the peripheral portions of the lobules. The portal areas are already thickened by proliferating cellular connective tissue and the number of small bile ducts is much more evident than in the normal animal. The lymph vessels in this location are distended with small clumps of dust-containing phagocytes.

The hepatic lymph node shows more marked reaction than the liver at this time. Its medullary sinuses are distended with extremely large giant cells packed with fine dust particles, visible only with polarized light. The afferent lymph vessels are so dilated that their diameter exceeds that of a low power microscopic field. They contain only coagulated lymph.

The next animal, killed at 9 months, shows more dust phagocytes in the sinusoids. Usually they occur in small clusters and are surrounded by a few lymphocytes. The portal connective tissues are now greatly increased in thickness at the expense of the parenchyma, but there is no demonstrable fibrosis within the lobules. At the junction of connective tissue and parenchyma cells there are numerous mononuclear phagocytes but no polymorphonuclear leukocytes. The fibrous tissue is cellular but edematous. The lymphatic spaces are widely dilated and in some places they contain clusters of large

dust-filled giant cells. In addition there are compact clusters of mononuclear phagocytes, frequently in mitosis, which are surrounded by concentric strands of compressed cellular fibrous tissue. These foci contain exceedingly large numbers of minute dust particles. In some instances these lesions have already been replaced by compact nodules of loose cellular connective tissue.

The next 2 animals were not killed until the 656th day, by which time the reaction had progressed to extreme limits. The portal connective tissues have increased tremendously and large groups of lobules have been completely destroyed and replaced. Some portions of the parenchyma appear never to have been involved, while others present evidence of regeneration after injury. The connective tissue is generally non-cellular in character and exhibits clusters of nodular whorls of hyaline fibers staining intensely with eosin. Compact masses of minute, doubly refractile dust particles can be found in the centers of such areas. Elsewhere the connective tissue consists of either heavy hyaline bands or loose edematous fibers. Scattered through it are clusters of small bile ducts and fragments of parenchyma varying in size from a few cells to the central half of a lobule. The liver cells are infiltrated with fat. No polymorphonuclear leukocytes are seen in or about them. Traces of lymphatics and sinusoids have disappeared, but in isolated spaces there are multinucleated, deeply eosinophilic cells, which are probably phagocytes as some of them contain dust particles. As evidence of regeneration there are areas of parenchyma in which no suggestion of lobular arrangement or sinusoids can be discovered. The cells in such areas are pale, non-granular and free from visible fat. No dust particles can be found among them. Uninvolved portions of the parenchyma are apparently normal, except for a well marked fatty infiltration rather sharply limited to the central portion of the lobule.

The hepatic lymph nodes in these animals are completely replaced by compact whorls of dense hyaline connective tissue.

The 4 remaining rabbits examined between the 677th and the 1067th day show only minor variations from the picture just described. Apparently the fibrosis continues to progress with increasing amounts of hyalinization, but some of the less dense connective tissue appears to have been replaced again by regenerating liver cells. In its older portions the fibrous tissue is absolutely devoid of nuclei but where it approaches large blood vessels, in the capsule for

example, it becomes markedly cellular with numerous mitotic figures. While all trace of sinusoids has disappeared there are occasional areas, apparently unconnected with large blood vessels, which are filled with living blood cells. The large bile ducts are preserved intact but evidence of bile stasis is furnished by scattered phagocytes filled with yellow-green pigment.

The gross picture is that of a coarse nodular cirrhosis with marked distortion of the organ. Areas 2 to 3 cm. in diameter may be composed wholly of white, leather-like scar tissue. Sometimes spherical nodules, 8 to 10 mm. in diameter, are attached to the surface of the organ by strings of fibrous tissue. In the less involved portions bright red areas of granular liver tissue are furrowed by irregular depressed bands of fibrosis. In the animals killed later the regenerated portions of the organ project as smooth, dark red masses elevated above the older, gray, nodular, cirrhotic areas.

Larger Silica Particles (6 to 12 Microns)

The wider variation in the size of the particles used in this group is responsible for involvement of different organs. The larger particles are caught in the pulmonary capillaries; some of the smaller ones are carried to the spleen and liver. Not enough are retained within the latter organ to provoke significant reaction, but they are removed by the lymphatics to the hepatic lymph node near the head of the pancreas.

Lungs: In the lungs large fragments of quartz are phagocytosed by mononuclear and giant cells, which collect within the thickness of the alveolar septa. By 180 days after the last injection they have formed nodules that vary in size from only a few cells to that of a distended alveolus. A few particles are transported to adjacent lymphoid tissues. At this time silver impregnation shows fine reticulum fibrils extending from the surrounding connective tissue between the mass of cells. In the course of years these nodules may increase in size and perhaps in number, but in the majority of cases the progression of such lesions is insignificant. In 1 of 3 animals killed after 3 years the nodules are somewhat larger and considerable numbers of dust-containing phagocytes are seen within the intrapulmonary and tracheobronchial lymphoid tissues. However, none of the lung nodules has developed any fibrous characteristics. The

phagocytic cells are still round and although the reticulum fibers are more abundant and somewhat thicker no collagen has developed.

Spleen: In the sinuses of the spleen the polarizing microscope shows considerable numbers of smaller quartz particles (6 to 8 microns). They are included within the cytoplasm of extremely large giant cells occurring in compact clusters within the sinuses. These nodules appear to undergo no further change during the maximum period of observation (1130 days).

Liver: In marked contrast to the extreme reaction provoked by the finer sized quartz dust of the first fraction there is practically none in the liver of any animal of this group injected with 6 to 12 micron particles. In gross the organ appeared perfectly normal. In microscopic sections there is an occasional giant cell containing a particle caught in a sinusoid, and very rarely a small cluster of such cells is found in the portal connective tissues. As time has progressed the only additional feature found is the appearance of a small number of lymphocytes about the dust-containing cells.

The hepatic lymph node shows much more extensive reaction. Most of the normal elements are replaced by wide sheets of large phagocytes. Moderately coarse dust particles are visible by polarized light. In one or two areas there are nodular collections of phagocytes surrounded by definite bands of collagen. In such foci there are a greater number of smaller dust particles (6 microns +).

Kidney: In neither group injected with silica has there been evidence of the dust or reaction to it in the kidney. If the foreign body reached this organ it was apparently not retained. In the case of the smaller fraction macroscopic nodules developed in the marrow of the ribs, which progressed to the stage of cellular connective tissue without hyaline formation.

Fine Aluminum Oxide

Eight rabbits were given 1.2 gm. of aluminum oxide particles, 1 to 3 microns in diameter, in from five to seven intravenous injections, during a period of $4\frac{3}{4}$ months. When killed, at intervals during the following 22 months, none of them showed gross evidence of actual disease. The only changes in any way suggestive of lesions were patches of pigmentation where the black dust particles escaped into the areolar tissues of the ears, at the site of injection, and a slightly increased prominence of the lobulation of the liver.

As in the case of quartz the particles are distributed throughout the various organs according to their size, but aluminum oxide has a very different effect upon the phagocytes and upon connective tissue. A limited number of phagocytes is adequate to care for the alumina injected because each cell ingests an excessive quantity of particles. So many of them accumulate within the cytoplasm that the internal structure of the phagocyte is often completely obscured. Under favorable conditions several nuclei can be made out within a cell hypertrophied to several times its normal size. Migration is apparently impeded as the giant cells remain scattered throughout the tissue and do not collect in masses as in the case of quartz. Furthermore, ingested alumina particles do not seem to injure the cells for there is no accumulation of polymorphonuclear leukocytes or proliferation of mononuclear phagocytes. Evidence of stimulation of fibroblasts has been entirely lacking throughout the entire period of observation. In fact the only further change observed in nearly 2 years after completing the injections has been a slight accumulation of lymphocytes about the alumina-containing cells.

In the *lungs* there are capillary emboli of dust particles surrounded by a few phagocytes. In the *spleen* isolated mononuclear or giant cells packed with black particles are scattered widely throughout the sinuses, follicles and interstitial tissues. In the *liver* there are giant cells in the sinusoids at the periphery of the lobules and in the portal connective tissues. Twenty-two months after completing the injections the only change that has resulted is a moderate degree of lymphocytic infiltration about these depots.*

Discussion

This experiment has provided new data on the biological activity of quartz particles of various sizes. It has also demonstrated the inability of aluminum oxide, a commonly used abrasive material, to provoke a corresponding reaction. To the experimental pathologist these observations are of interest for two reasons: (1) they demonstrate that insoluble foreign particles injected into the blood stream are filtered out by various organs according to their size, and (2) they

* Similar experiments have been made with *diamond dust*. The quantity injected was less than in the case of silica and alumina but 1 of 5 animals was allowed to survive for 3 years. The distribution and reaction to these extremely hard particles were in every respect comparable to that produced by alumina.

illustrate the capacity of extrapulmonary tissue to react to silica in a characteristic manner. The silicotic cirrhosis produced in the liver is a curious lesion. To the industrial hygienist the contrast between the rapidly progressive reaction produced by very fine quartz particles and the sluggish reaction to coarser quartz and aluminum oxide is of practical significance.

The capacity of the tissues to filter out and localize particles of different sizes in various organs has been the factor determining the distribution of the disease produced by the injected silica. The largest dust particles were apparently retained mechanically in the capillaries of the lungs. Somewhat smaller ones were held back in the spleen while the finest ones were lodged in the liver. The lymphatics of this organ removed as many as possible to its lymph node, situated near the head of the pancreas adjacent to the common bile duct and portal vein. The large number of fine particles provoked such a severe reaction in the node that its function was disturbed; the flow of lymph in the afferent vessels was impeded and the foreign bodies could no longer be eliminated. As a result they accumulated in and about lymph vessels within the liver. This observation constitutes another instance of the importance of the lymphatic system in the development of silicosis. It is of interest to note that whereas in the pulmonary apparatus an obstructive lesion in the tracheobronchial lymph nodes is followed by accumulation of dust in the intrapulmonary lymphoid tissue, in the hepatic system, where there is normally no lymphoid tissue within the viscus, the dust particles accumulate in and about lymphatic vessels. The absence of dust particles and resultant reaction in the kidney is indicative of the lack of a specific mechanism for segregating particulate matter in this organ. These observations also substantiate the conclusions drawn from the inhalation experiments that the reaction to silica is a purely local one about points where particles have been concentrated and that no dissolved silica circulates in the blood stream to damage remote organs. These findings may be of clinical significance because of the disputed contention that the incidence of nephritis is excessive among the victims of silicosis.

A comparison of the reaction to fine quartz with that to aluminum oxide particles of the same size demonstrates the marked difference in the behavior of the phagocytes. The same cell will continue to ingest alumina particles until it becomes so overloaded that its struc-

ture is completely invisible and its power of locomotion is apparently lost. In the case of silica comparatively few particles are usually ingested by any one cell; wherever the number of very fine particles is excessive the cells hypertrophy and form huge giant cells. Either because there is no overloading and no mechanical interference with ameboid activity, or because the irritating particles stimulate migration, the silica-containing cells tend to collect in aggregates. Cells containing alumina, on the other hand, remain more scattered throughout the tissue. This difference in the distribution of the phagocytes ingesting the two types of dust has been maintained throughout the 2 to 3 year observation period of this experiment, and it has been repeatedly observed in other investigations where comparative reactions to quartz and other materials were being studied. It appears to be one of the fundamental factors in determining the outcome of the various forms of pneumonoconiosis.

The importance of the factor of particle size in producing reaction to silica is the chief contribution of this experiment. It has been shown that large particles, 10 to 12 microns in diameter, provoke a foreign body reaction in the lungs which persists without much further change for nearly 3 years. Smaller ones, from 3 to 6 microns in diameter, cause slowly progressive changes in the spleen and hepatic lymph node. Very fine ones of the order of 1 micron in size result in progressive proliferation of connective tissue in the liver.

It might be objected that the number of large particles localizing in any one area was not sufficiently great to provoke reaction, but this objection is met by the findings in the spleens illustrated in Figures 11 and 12. It will be noted that the relatively large number of particles, 6 to 8 microns in diameter, have provoked only a reaction of phagocytes 3 years after their injection, whereas the much smaller number of fine particles, 2 to 3 microns in diameter, have caused an extensive proliferation of connective tissue. It therefore seems justifiable to conclude that fibroblastic proliferation occurs because of the surface area and not because of the mass of silica in a given area.

Space does not permit a discussion of the histogenesis of the silicotic nodule but this subject has already been covered in the previously mentioned paper on silica inhalation.[1] It may be stated here that the original nodule of phagocytes, formed by migrating cells, increases in size as a result of mitotic division. The silica concen-

trated in the center of the mass kills many of the cells and this excites a moderate amount of leukocytic infiltration. At the same time fibroblasts are growing into and replacing the original mass, so that it now comes to resemble an actively growing fibrosarcoma. After a year or more this connective tissue becomes fibrous in character and finally a characteristic hyaline swelling takes place at the center, involving one fiber after another. Since such lesions often contain patent blood vessels it is believed that hyaline formation is due to the toxic action of silica rather than to lack of nutrition.

Cirrhosis of the liver developed in the group of animals injected with very fine quartz dust because sufficient quantities of active silica particles were caught and retained in its stroma. No cirrhosis resulted from the larger quartz particles because most of them were removed by the lymphatic system and the few that remained were too large to be active. No cirrhosis was produced by the injection of the same quantity of fine aluminum oxide particles because they are incapable of exciting a progressive fibrosis.

The part played by the phagocytes and the lymphatic system in the concentration of silica particles has already been discussed. The presence of a sufficient quantity of this irritant in the lymphatics of the portal connective tissues set in motion a progressive proliferation of fibroblasts in their immediate vicinity. Increase of the stroma took place at the expense of the parenchyma of the organ until large portions of the functioning elements had been replaced. Whether the liver cells were destroyed wholly as the result of pressure or whether they were first injured by the toxic action of the silica is not clear. There was marked fatty infiltration, but in only one animal, killed on the 980th day, was there any marked infiltration with polymorphonuclear leukocytes. In the inhalation experiments guinea pigs, but not rabbits, exhibited a well marked metastasis of inhaled dust to the liver and in this location there was a well marked leukocytic infiltration. In these animals injected intravenously only mononuclear leukocytes were found between the advancing edge of the fibrous tissue and the parenchyma. Whether leukocytic infiltration is a feature of the process which was not observed because the animals were not killed at proper intervals is not known.

The overgrowth of fibrous tissue between and within the liver lobules resulted in an extreme grade of coarse nodular cirrhosis of macroscopic proportions. It was apparently much more marked

than the lesion that Gye and Purdy produced with intravenous colloidal silica, but the amount of silica introduced in our experiments was greater and the animals survived considerably longer. The later stages of the picture were further complicated by regeneration of extensive portions of the parenchyma. This furnishes some extremely bizarre gross pathological pictures. Apparently a sufficient portion of the organ continued to function in most instances to maintain the circulation, as only 1 rabbit developed ascites. This was in an animal that died on the 656th day.

Summary and Conclusions

Three series of rabbits were injected intravenously with 1.3 gm. respectively of silica particles 1 to 3 microns in diameter, silica particles 6 to 12 microns in diameter, and aluminum oxide particles 1 to 3 microns in diameter. The injections were given in divided doses and required from 1 to 4 months for their completion.

These particles were segregated in different locations according to their size. The largest ones were caught in the pulmonary capillaries, those of intermediate size in the spleen and hepatic lymph node, and the finest ones in the liver.

Fine silica particles are most active and have produced a progressive, coarsely nodular cirrhosis of the liver attended by extensive destruction of the parenchyma, followed later by regeneration in certain areas. This cirrhosis is the result of a typical, hyaline, nodular, silicotic fibrosis originating in the portal connective tissues.

Coarse silica particles, 10 to 12 microns in diameter, are much less irritating. They excite a simple foreign body type of reaction which has progressed very little in practically 3 years time.

Fine aluminum oxide particles of the same size as those of the smaller silica fraction and introduced in essentially the same quantity have been merely phagocytosed and have produced no fibrosis in the stroma of any organ where they were deposited.

These observations support the viewpoint that the injury produced by silica is specific and chemical rather than physical in character.

REFERENCES

1. Gardner, L. U. Studies on experimental pneumonokoniosis. VIII. Inhalation of quartz dust. *J. Indust. Hyg.*, 1932, **14**, 18–38.

2. Collis, E. L. Industrial pneumonoconioses. With special reference to dust-phthisis. Milroy Lectures, 1915, H. M. Stationery Office, London.

3. Gye, W. E., and Purdy, W. J. The poisonous properties of colloidal silica. I. The effects of the parenteral administration of large doses. *Brit. J. Exper. Path.*, 1922, **3**, 75–94.

4. Heffernan, P. Acute silicosis. *Tubercle*, 1932, **14**, 109–112.

5. Cummings, D. E. Studies on experimental pneumokoniosis. IV. The separation of particulate matter smaller than screen sizes into graded fractions. *J. Indust. Hyg.*, 1929, **11**, 245–256.

DESCRIPTION OF PLATES

PLATE 120

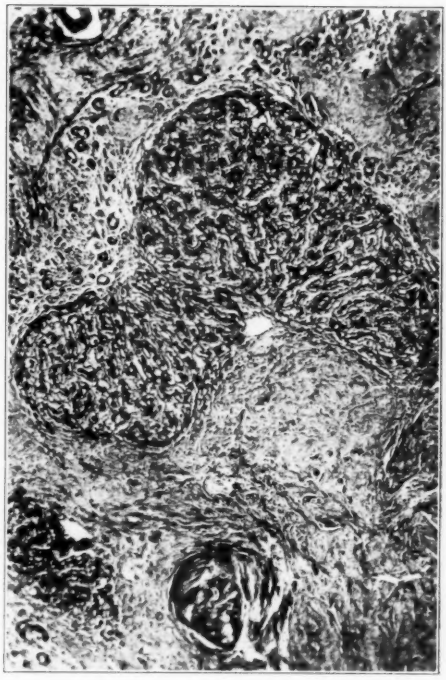
FIG. 1. Silicotic cirrhosis of the liver. Animal killed 1067 days after completing injections of silica. Upper portion shows nodular external surface of organ. Lower portion shows cut surface. Note large nodule composed wholly of scar tissue in the center. × 1.66.

FIG. 2. Silicotic cirrhosis produced by 1 to 3 micron silica particles. Animal killed at 655 days. Only a portion of several liver lobules is left in the center of an extensive fibrosis. × 100.

FIG. 3. Spleen of rabbit injected with 1.2 gm. alumina particles 1 to 3 microns in diameter. Killed in 656 days. Note the lack of reaction about dust-filled phagocytes. × 150.

FIG. 4. Liver of rabbit injected with 1.2 gm. alumina particles 1 to 3 microns in diameter. Killed at 1095 days. Compare with Fig. 2, showing reaction to the same amount of the same sized silica particles. × 150.

1



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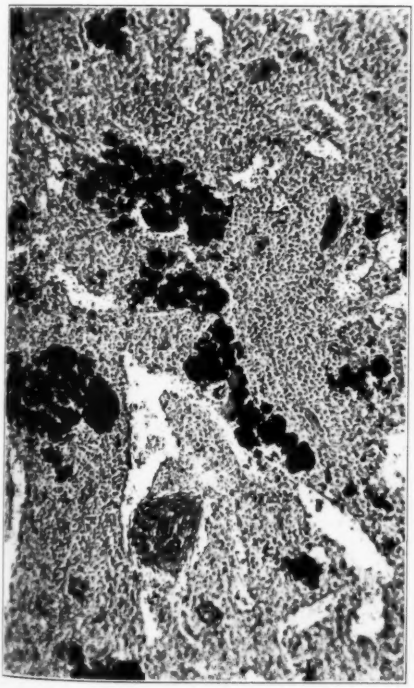

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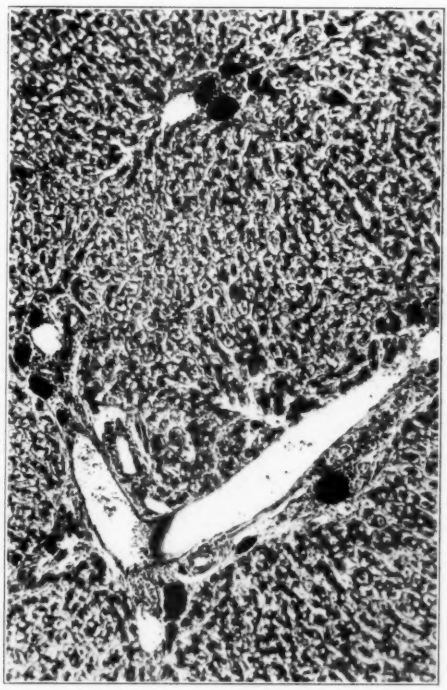


4

Gardner and Cummings

Reaction to Quartz and Aluminum Oxide Particles

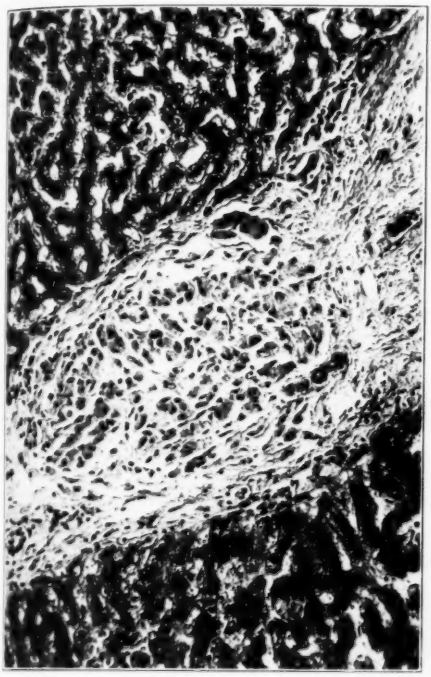
PLATE 121

FIG. 5. Nodule of dust phagocytes within a band of beginning fibrosis in the portal area. Animal injected with 1 to 3 micron silica particles. Killed after 275 days. × 200.

FIG. 6. Hyaline transformation in a nodule similar to that shown in Fig. 5. × 200.

FIG. 7. Extensive nodular hyaline fibrosis in portal area. The centers of the nodules are filled with fine dust. No nuclei are left. Animal killed after 655 days. × 100.

FIG. 8. Regenerated parenchyma in a silicotic cirrhosis. Animal killed 677 days after injections. × 100.



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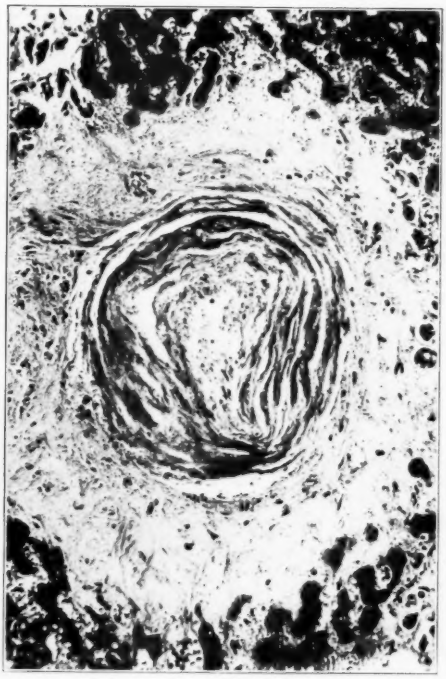

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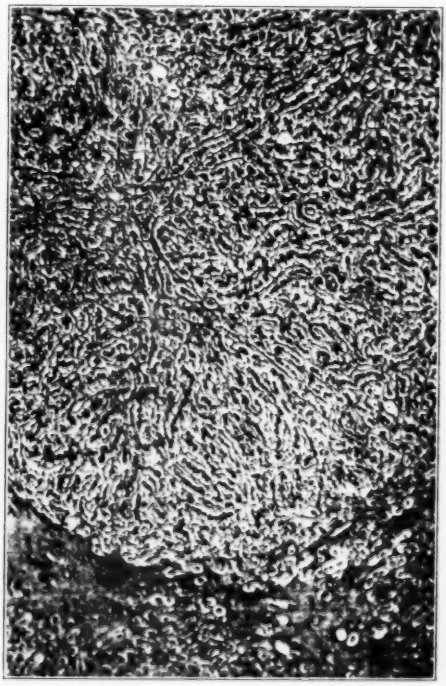


8

Gardner and Cummings

Reaction to Quartz and Aluminum Oxide Particles

PLATE 122

FIG. 9. Lung of rabbit killed 180 days after the injection of 1.3 gm. of 6 to 12 micron silica particles. Note the foreign body giant cell reaction with a minimum amount of reticulum. Foot's silver impregnation method. × 200.

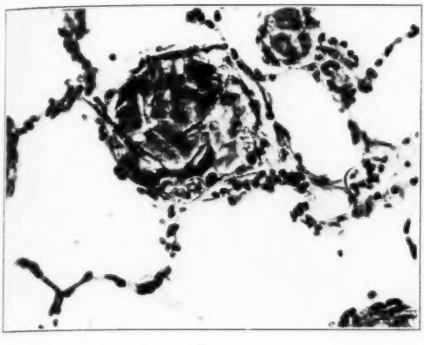
FIG. 10. Another animal of the same series shown in Fig. 9, but not killed until 1130 days after injection. Note the relatively slight increase in size of the nodule and of the amount of reticulum. × 200.

FIG. 11. Spleen of rabbit killed 1067 days after injection of 1.3 gm. of 1 to 3 micron silica particles. Fibrous nodule with very early hyalinization of a few fibers is seen. This reaction is progressive. × 95.

FIG. 12. Spleen of rabbit injected with the same quantity of silica particles 6 to 12 microns in diameter and killed 1130 days after injection. This reaction is non-progressive and consists only of nodules of phagocytes. × 95.

FIG. 13. Silica particles from nodule shown in Fig. 11, seen by polarized light. × 240.

FIG. 14. Silica particles from nodule shown in Fig. 12, seen by polarized light. × 240.



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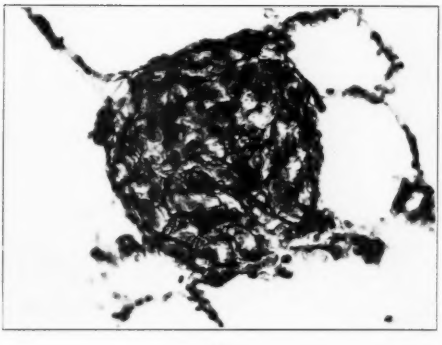

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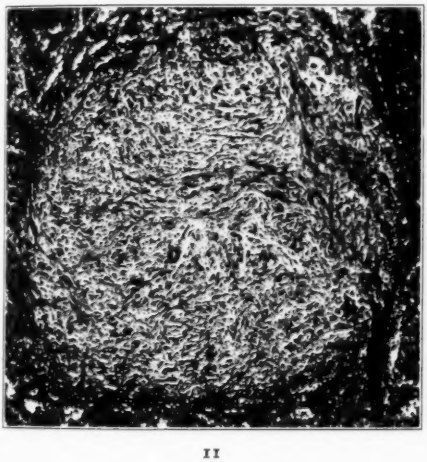

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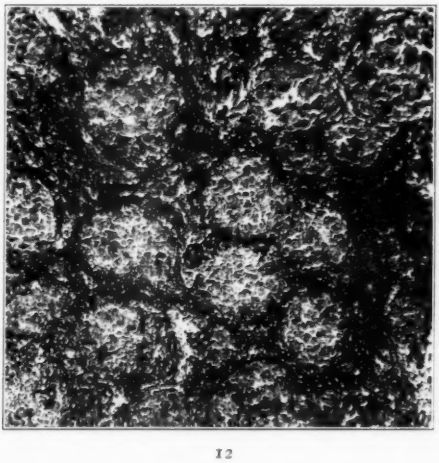

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Gardner and Cummings Reaction to Quartz and Aluminum Oxide Particles

A GROUP OF METAPLASTIC AND NEOPLASTIC BONE- AND CARTILAGE-CONTAINING TUMORS OF SOFT PARTS *

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The occurrence in soft tissues of tumor-like masses containing bone and cartilage has been recognized since the time of Rokitansky. However, the origin of these structures — more particularly of the extensive and frequently dramatic ossifications of muscle following trauma — has been the subject of an immense volume of speculation, of clinical and pathological observation and of ingenious experimentation, which has recently been well summarized by Koehnlein.[1] Three theories appear worthy of consideration.

1. Cahen [2] in 1891 suggested a neoplastic origin. Though certainly to be discarded in the great majority of cases of ossification in muscles, tendons and ligaments, it must be granted as established in such cases as the frankly malignant, bone-containing neoplasm reported as Case II by Rhoads and Blumgart.[3]

2. Origin from periosteum may well explain the type of ossification of tendons, usually described as spur formation, which is always directly connected with bone and usually follows injury or exceptionally heavy functional strain. Examples of this type showing spur formation from the olecranon into the triceps tendon in tennis players and boxers have been described by Baetzner.[4]

Periosteal displacement has been and still is warmly advocated as the cause of so-called myositis ossificans, and the less well known tendonitis ossificans, by many authorities from Berthier [5] and Lexer [6] down to the present day. Clinical arguments in its support are the frequency of preceding trauma, the points of predilection in muscles with large bony attachments, and the frequent roentgenographical evidence of periosteal involvement. The experiments of de Josselin de Jong and Eykman van der Kemp,[7] and many others, in transplanting bone and periosteum into the soft parts offered apparent confirmatory evidence. Nevertheless, all possibility of

* Received for publication May 1, 1933.

periosteal displacement can be ruled out in many instances. This is particularly true for instance of the numerous cases of ossification of laparotomy wounds, such as the one carefully described by Gruber,[8] and of the cases of congenital generalized myositis ossificans in children, where clinical palpation of the tissues has been followed by ossification.

3. Origin by metaplasia from fibrous tissue was first suggested by Virchow,[9] who believed that all bone and cartilage — both normal and abnormal — arose by metaplasia from fibroblastic elements. He regarded osteoblasts and chondroblasts merely as modified fibroblasts. This view, though never universally accepted, has always had its warm proponents — notably Mallory,[10] and still more recently Leriche and Policard.[11] So far do the latter authors swing the pendulum in applying the doctrine of metaplasia to bone and cartilage formation that they regard osteosarcomas merely as fibrosarcomas passively ossified by the local conditions of their environment. In favor of this view may be cited the ossification occasionally seen in leiomyomas, neurofibromas and colloid adenomas of the thyroid. Not only does metaplasia offer a simple and satisfactory explanation for the ossification of muscle, tendons and ligaments, but it appears to be the only possible explanation of the many other types of heterotopic bone formation constantly met by the pathologist, such as ossification of arteries, heart valves, of pericardium and pleura, of obsolete tuberculous foci, or of the falx cerebri, to mention only the commoner types.

From clinical and pathological observation metaplastic bone formation can fairly definitely be divided into two types. In one, frank and often long-standing calcium deposits are slowly organized into bone by the fibroblasts of the immediately adjacent tissue. In the other, the formation of cartilage or osteoid tissue either precedes or occurs simultaneously with the deposit of the calcium, as in many cases of myositis ossificans. The mode of ossification in this type parallels with great fidelity normal bone formation in intra- and extra-uterine life, and like it may be either direct from fibrous tissue or secondary to cartilage formation. That combinations of the two types might occur it would be rash to deny.

Experimental production of both types has been successful. The ligation of the renal artery in rabbits is followed by calcification of the degenerating renal tissues and subsequent ossification (Sacer-

dotti and Frattin,[12] Liek,[13] and others). Ossification of the second type has been achieved by Gruber [14] through traumatization of muscles in rabbits, and by Leriche and Policard [15] in especially constructed tubes of granulation tissue. These experimenters obtained their greatest success if at the same time they produced an experimental destruction of bone in an adjacent area. They feel that two factors are necessary for metaplastic bone formation — young, actively proliferating fibroblasts, which have retrogressed toward the embryonic state, and an excessive supply of calcium, which an adjacent focus of bone necrosis supplies.

From the point of view of differential diagnosis, clinical, X-ray or histological, lesions of the first type rarely offer difficulty. In contrast, lesions of the second type, if caught at the stage of active fibroblastic proliferation, before extensive calcium deposits have occurred, may be extremely difficult to differentiate from neoplasms, either benign or malignant. The following group of cases includes various stages and types of chondrification and ossification in soft tissues, both metaplastic and neoplastic, which offered problems of diagnosis and interpretation sufficiently interesting to seem worthy of record.

Case Reports

Case 1.* *Clinical History:* R. H., female, aged 28 years, unmarried, in good general health, had noted occasionally for the preceding year a peculiar tired feeling in the right hand after writing or exercise. Three or four weeks before entry tenderness developed over the right hypothenar space. Flexion of the fourth and fifth fingers gradually became so painful that she could not use her hand and it was immobilized by a physician. The condition became steadily worse, the tenderness and swelling over the palm increasing, and swelling appeared also on the dorsum of the hand between the fourth and fifth metacarpals. Pain was severe enough to keep her awake at night and to require morphia.

The past history was negative except for a compound fracture of the right elbow four years before, which had drained for several weeks before healing. The hand had not been injured at that time.

The physical examination was negative, except for the local findings. The ulnar third of the palm, particularly over the region of the hypothenar space, showed moderate redness, increased warmth, marked swelling and acute tenderness. Active or passive flexion of ring and little fingers was limited. There was slight swelling and tenderness on the dorsum of the hand between the fourth and fifth metacarpals.

X-ray examination one week after onset was entirely negative. At the time of entry there was evidence of soft tissue swelling. One small linear shadow

* Case of Dr. Arthur W. Allen.

medial to the upper extremity of the fifth metacarpal was noted but was thought to have no pathological significance. The bones and joints showed no evidence of disease.

In the hospital, before operation, a slight fever was noted, reaching 99.5° to 100° F every day. White counts were 12,400, 14,900 and 7400. The differential count showed 63 per cent polymorphonuclears. Since no improvement was noted after six days of complete rest Dr. Allen decided to explore. His preoperative diagnosis was infection of the hypothenar space. Incision was so made as to expose the ulnar nerve over the hypothenar space. A gristly, firm, friable, tumor-like tissue was found filling the space and extending downward into the region of the interosseous muscles between the fourth and fifth metacarpals. A frozen section was made during the operation, which showed an extremely cellular tissue containing both fibroblasts and histiocytes. Mitoses could be identified. Hyaline material thought at the time to be atypical cartilage was present. Although the findings suggested sarcoma this diagnosis was at such variance with the clinical picture that it was not felt warranted and diagnosis was withheld until after examination of the fixed tissue. Since complete resection was impossible without injury to the ulnar nerve and a mutilating and crippling operation, no attempt was made to remove all the material and approximately one-third of it was left undisturbed.

Upon receipt of a diagnosis of probable myositis ossificans from the histological examination of the fixed tissues all thought of further operative interference was abandoned. The local symptoms rapidly cleared. A small tumor was still palpable, which gradually became firmer. X-ray examination six weeks postoperatively showed a sharply circumscribed, densely calcified mass in the region of the persisting tissue. One and a half years later there had been no further change.

Microscopic examination of the Zenker-fixed tissue showed a complex mixture of tissues including degenerating, multinucleated skeletal muscle cells, well formed but non-calcified lamellae of osteoid material, irregular sheets of osteoid enclosing scattered, large, stellate cells, spindle-celled areas of high cellularity with fairly numerous mitotic figures, and large areas rich in collagen where the collagenous bundles were separated by chains of cells characteristic of tissue histiocytes rather than fibroblasts. These areas were histologically similar to some of the less typical benign giant cell tumors of tendon sheaths. They resembled still more closely the granulomatous tumor masses seen in periarticular tissues. No foam cells or giant cells were present, however. Direct formation of lamellar bone from osteoblasts, which show all stages of transition from fibroblasts, was particularly evident and is illustrated in Figures 1, 2 and 3.

Comment: The absence of trauma, the local signs of inflammation, the slight temperature and elevated white count were all indicative

of an inflammatory lesion. The operative finding of tumor-like tissue was a great surprise and the results of an attempted frozen section diagnosis were inconclusive. Even with paraffin sections of the fixed tissues many areas were so suggestive of sarcoma that numerous pathologists who have reviewed the slides have felt unable to rule out malignancy and some were frankly in favor of it.

It was particularly fortunate that there were good reasons for leaving a portion of the growth undisturbed. The subsequent complete calcification of this remnant, the cessation of growth and even slight shrinkage would appear to rule out beyond reasonable doubt malignancy and even benign neoplasia. The similarity of the histological picture to that of Case I, reported by Rhoads and Blumgart, would raise in the author's mind the question whether that case also was not one of metaplasia rather than neoplasia.

As a case of proved myositis ossificans it is interesting in that the successive X-rays demonstrate clearly the absence of significant deposits of calcium until after a lamellar framework of osteoid material had been laid down. An excess of calcium can hardly have been the stimulating factor that initiated the process.

CASE 2.* *Clinical History:* E. A., female, aged 28 years, four months before entry noticed a "bunch coming up overnight" on the dorsum of the left foot. She could remember no injury. During the interval the mass grew but slightly and was painful only occasionally.

Examination showed a hard, fixed mass over the third and fourth metatarsals of the left foot. It was not tender and the skin over it was not red. An X-ray plate made one month before admission showed a barely visible, noncalcified tumor mass that appeared to cause slight mechanical spreading of the metatarsals. X-ray examination on admission to the hospital showed definite calcification in the tumor and also two small calcified masses apparently unconnected with the tumor but lying near it between the same pair of metatarsals.

At operation, incision through the skin, subcutaneous tissue and some unidentified dense fibrous tissue, exposed a tumor mass about 2 cm. in diameter lying on and apparently arising from the sheaths of the flexor tendons between the third and fourth metatarsals. It was about 2 cm. in diameter, firm, in part bony, in part cartilaginous, and was readily resected. Convalescence was uneventful.

X-ray examination seven weeks later showed a persistent small area of dense calcification between the metatarsals. A year later this same shadow was present, no larger but now showing a bony pedicle connecting it with the third metatarsal.

Histological examination elsewhere was reported as probable osteogenic sarcoma. Histological examination of two slides referred

* Case of Drs. J. W. Elliot and Harold F. Miller.

to us showed the bulk of the tumor to consist of connective tissue, in part densely collagenous, in part myxomatous, extremely cellular, with occasional mitotic figures. The cells between the collagen bundles were in part typical fibroblasts, but many others were unusually large and blunted, with irregular, oval to horseshoe-shaped nuclei and abundant cytoplasm. These often resembled closely or were indistinguishable from histiocytes. Transitional stages from the myxomatous areas into atypical fibrocartilage and from the more densely collagenous areas into hyaline osteoid material were evident. Several trabeculae of well formed, calcified bone were present. No muscle cells and no giant cells were seen.

Comment: This case also showed histologically active bone formation and areas of fibroblastic proliferation with mitotic figures. In addition there were large masses of rather poorly differentiated fibrocartilage. The first impression was that we were dealing with a slowly growing osteogenic sarcoma. Once again, however, the presence of numerous histiocytic elements did not fit well with a neoplastic origin. Multinucleated cells of the tumor giant cell type could not be found. The location ruled out a muscular origin, but lesions indistinguishable from myositis ossificans do occur in tendons and are often classified as tendonitis ossificans. Since, however, the mass was distinctly outside the tendon it seemed more logical to regard it as metaplastic cartilaginous and osseous transformation of one of the granulomatous tumor-like nodules of tendon sheaths. Once again the successive X-rays showing no calcification, slight calcification, and later dense calcification of the persisting remnants prove that the deposit of the framework of cartilage and osteoid preceded the deposit of calcium.

Case 3. *Clinical History:* K. M., female, aged 61 years, a paranoiac, with extensive hypertrophic arthritis, varicose veins and a ventral hernia. For two years a tumor had been present over the anterior aspect of the proximal phalangeal joint of the fourth finger. X-ray examination showed it to be calcified and to lie in the soft tissues without demonstrable connection with bone. During the two year period of examination it grew very slightly and its X-ray appearance did not change.

It was resected without difficulty under local anesthesia and was found to overlie and involve the tendon sheath of the flexor hallucis longus.

Gross examination showed a pinkish white, partly cartilaginous, partly friable and granular mass measuring 2.7 by 2.5 by 2 cm.

Microscopic examination showed the mass to consist wholly of

cartilage of varying degrees of differentiation with extensive deposits of calcium. No trace of ossification was discovered (Fig. 4).

Comment: The location of this tumor was characteristic of a tendon sheath granuloma. The frequency of myxomatous degeneration in such lesions is well known and the possibility of metaplastic organization into cartilage is recognized. It seems more logical to regard this as a chondromatous metaplasia than as a primary chondroma. It is interesting that in spite of the extensive calcification of the cartilage neither of the two reactions to calcified cartilage usual under normal conditions — ossification or foreign body giant cell reaction — had occurred.

CASE 4. *Clinical History:* M. B., female, aged 17 years, a previously healthy girl entered the hospital complaining of a "wart" of three years duration on the volar surface of the right hand, overlying the metacarpophalangeal joints of the second and third digits. She had been treated for verruca vulgaris, presumably in acceptance of her own diagnosis, with intramuscular salt solution and bismuth injections (not in the neighborhood of the lesion) without effect.

Examination showed a subcutaneous swelling covered by thickened, callus-like skin. A preoperative diagnosis of cutaneous fibroma was made. Resection was done under local anesthesia and a nodule 1 by 1 by 0.7 cm. with the overlying skin was removed. On section it appeared to consist of bone with a few areas suggestive of cartilage.

Microscopic examination showed on one surface of the tumor pacinian corpuscles and coil glands, and on the other tendon. The bulk of the tumor consisted of trabeculae of well formed bone separated by a vascular stroma containing numerous osteoclasts. At the periphery of the lesion were a few foci of hyaline cartilage. There was no evidence of rapid growth.

Comment: This tumor was so superficial that it lay in part actually within the skin. At its base it backed upon the tendon sheath. The possibility of an extruded sesamoid must be considered but in the absence of any history of present or past functional difficulty in moving the fingers it seems improbable. The histological appearance would suggest that the ossification here was preceded by chondrification and that this process represents the end stage of the same condition described in Case 3.

CASE 5.* *Clinical History:* I. I., female, aged 65 years. For a period of two years the patient was aware of a slowly growing, non-painful tumor of the left thigh. In August, 1929, she consulted a surgeon, who excised the tumor. A

* Case of Drs. S. B. Overlock and R. B. Greenough.

pathological diagnosis of "giant cell tumor" was made upon histological examination. A few weeks later the wound broke down and the tumor recurred locally.

She was seen by Dr. Greenough in January, 1930. At this time he found a large tumor mass, bluish in color, involving the anterior and lateral aspect of the left thigh and extending nearly to the groin, where there were several hard nodes. The abdominal examination was negative.

The possibility of a successful amputation seemed very slight and operation was not advised. By March, 1931, the tumor had broken through the skin, was ulcerated, and had given rise to severe hemorrhage. The patient was losing weight and failing rapidly. The date of her death has not been ascertained but must have been soon after this.

A microscopic slide was sent to us prepared from the original tumor mass. It showed an unencapsulated tumor that was invading the fat tissue at its periphery. Considerable portions of it were necrotic. In the central portions several old calcified areas, some of them strongly suggestive of necrotic bone trabeculae, were observed. The major portion of the viable part of the tumor consisted of masses of giant cells embedded in a cellular, highly vascular tissue composed of spindle cells, apparently typical fibroblasts. Numerous mitotic figures were present in these cells. The giant cells were variable in size, ranging from typical foreign body giant cells with twenty to thirty nuclei to small cells with scanty cytoplasm and three to four nuclei, indistinguishable from tumor giant cells. In occasional areas, particularly in the immediate neighborhood of the old calcium deposits, but also at some distance from them, irregular trabeculae of hyaline osteoid material were found.

Comment: Although the general appearance of this tumor with its immense numbers of foreign body giant cells closely resembled a benign giant cell tumor the frankly sarcomatous nature of the spindle cell masses in which the giant cells were embedded, the presence of cells indistinguishable from tumor giant cells, the rapidity of growth and the invasiveness appeared to leave no question of the present malignant neoplastic nature of the mass. Two possibilities of origin seem reasonable. A fibrosarcoma may have arisen in or near a mass of calcium, possibly an old focus of myositis ossificans or a calcified hematoma. The bone formation might be considered a metaplasia of the fibroblastic tumor elements influenced by the local presence of calcium. This would fit well with the theories of Leriche and Policard. The foreign body giant cells might easily be accounted for by the unorganized calcium masses. The other alternative is to re-

gard the tumor as an osteogenic sarcoma of soft tissues, either primary or possibly secondary to a much older metaplastic process.

The clinical course of the lesion, with the recurrence and the metastasis to the inguinal nodes, leaves no doubt of its malignancy.

CASE 6.* *Clinical History:* S. K., female, aged 55 years. Three months before visiting her physician the patient noticed for the first time a mass in the left breast. It had been painless but it grew rapidly, and by the time she consulted a physician the mass filled the entire breast. There had been no trauma. The skin over the lower aspect of the breast was reddened and at one point fluctuant over an abscess cavity that extended into the tumor.

A preoperative diagnosis of sarcoma of the breast was made and a simple mastectomy performed. The diagnosis was confirmed by frozen section. A radical mastectomy was not thought to be indicated. Convalescence was uneventful. One year later the patient was in good health with no evidence of recurrence.

Gross examination of the excised specimen showed a bulky, spherical tumor 10 cm. in diameter, well circumscribed but not encapsulated, with extensive, central, yellow to white necrosis. There was considerable calcification and a narrow peripheral zone that was pinkish, dotted with yellow and bright red areas, slightly translucent and soft.

Microscopic examination of the viable portions of the tumor showed a highly vascular and cellular mass of spindle-shaped cells, embedded in which were numerous large giant cells of the foreign body type containing ten to fifty nuclei. No cells characteristic of tumor giant cells were found and though numerous mitotic figures were present none of them were multiple. The spindle cells showed well marked fibroglia fibrils with the phosphotungstic acid hematoxylin stain. In many areas in the tumor well formed lamellae of hyaline osteoid material were present and all transitions from typical spindle-shaped fibroblasts to characteristic cuboidal osteoblasts plastered against the freshly forming osteoid could be observed.

Scattered throughout the tumor, but most numerous in the central necrotic zone, were small and large calcium deposits. Some of these were in direct relation to the osteoid material, others were not. Also scattered irregularly throughout the tumor were foci of hemorrhage, deposits of orange-brown pigment readily identified as hemosiderin and also masses of a much brighter yellow crystalline pigment. This latter pigment was suggestive of bilirubin in appearance and color

* Case of Dr. M. P. Smithwick.

and was readily oxidizable to a dark green shade with ferric chloride and trichloracetic acid (Fouchet's reagent for the identification of bilirubin).

Comment: This tumor, identical in histological appearance with that of Case 5, except for less obvious invasive qualities and somewhat better developed osteogenesis, is, so far as we have been able to ascertain, a unique observation in breast tumors. Though less frankly malignant than that in the preceding case the sarcomatous character of the basic fibroblastic masses, the rapidity of growth and the extensive necrosis, all indicate a neoplasm of indubitable malignancy.

Embedded in this tumor were numerous unorganized calcified masses and scattered granules. It seemed scarcely credible that they were the product of the tumor. A more reasonable view would be that at some period in the past calcification had occurred in the breast as the result of forgotten trauma, or of mastitis with fat necrosis, or of calcification of an adenofibroma. In the neighborhood of this old calcified lesion a fibrosarcoma developed. With increased vascularity partial absorption of the calcium occurred, stimulating a giant cell reaction and also the deposit of osteoid by the embryonic fibroblasts of the tumor.

Discussion

Cases 1 and 2 are particularly interesting because of the similarity of the histological picture, although Case 1 obviously was derived from the interosseous muscles of the hand, and Case 2 from a tendon sheath without involvement of muscle. Both showed sufficient evidence of rapid growth and sufficient atypicality of osteogenesis to be histologically strongly suggestive of neoplasm. In each case a succession of X-rays showed clearly that the active phase of metaplasia occurred before the deposition of significant quantities of calcium, and the source of the stimulus to metaplasia must hence be sought elsewhere.

Cases 2, 3 and 4, because of their attachments to the exterior of tendon sheaths in regions where the so-called xanthomas of tendon sheaths characteristically occur, appear most logically to be explained as phenomena of metaplasia in these nodules. In all of these there was evidence of a cartilaginous transformation preceding ossification. In fact, in Case 3 no ossification whatever was present,

although the cartilage was extensively calcified. In Case 4 at only a few areas on the edge of the tumor did a persisting rim of cartilage give evidence of its earlier stage.

Cases 5 and 6, essentially similar in histological structure, though occurring respectively in the thigh muscles and in the breast, both showed in the central portions of the tumor masses of unorganized calcium presumably antedating the appearance of the neoplasm or at least its active phase of growth. Although in each instance the presence of innumerable huge foreign body giant cells lent to the tumor an appearance almost convincingly similar to that of a benign giant cell tumor of bone, the character of the fibroblastic mass in which the giant cells were embedded was in each instance frankly neoplastic and malignant. The presence of lamellar deposits of osteoid material bordered in many instances by typical osteoblasts raised the question of considering the tumors as true osteogenic sarcomas of soft part origin. On the other hand, the findings fitted perfectly with the theory of Leriche and Policard that bone formation in tumors is essentially metaplastic, the requisites, just as in non-neoplastic metaplasia, being available calcium and embryonic fibroblasts. The old calcium deposits would supply the first, the tumor cells the second. We feel that these two cases present evidence suggestive of the correctness of their theory, as applied to at least a certain group of the extraskeletal bone-forming neoplasms.

Summary

The neoplastic, periosteal and metaplastic theories of extraskeletal bone formation have been discussed and illustrative cases presented. The difficulties of distinguishing between metaplastic and neoplastic lesions have been pointed out and reasons given for regarding Cases 1 to 4 as purely metaplastic and Cases 5 and 6 as primarily neoplastic. The possibility of metaplastic processes within neoplasms, suggested by Leriche and Policard as applicable to all tumors of bone, has been shown to be consistent with findings in these two cases.

Conclusions

1. Many apparent bone- and cartilage-containing tumors of soft parts are in reality of metaplastic rather than neoplastic origin.
2. Extreme care may be requisite to differentiate the active stage

of osteoblastic or chondroblastic metaplasia from malignant neoplasia.

3. Certain bone-forming spindle cell sarcomas of soft parts may more logically be regarded as fibrosarcomas with metaplastic foci of osteogenesis than as true osteoblastomas.

REFERENCES

1. Koehnlein, H. Knochenbildung in Sehnen. *Arch. f. klin. Chir.*, 1931, **163**, 147–172.
2. Cahen, F. Über Myositis ossificans. *Deutsche Ztschr. f. Chir.*, 1891, **31**, 372–382.
3. Rhoads, C. P., and Blumgart, H. Two osteoblastomas not connected with bone, histologically identical with osteogenic sarcoma, and clinically benign. *Am. J. Path.*, 1928, **4**, 363–369.
4. Baetzner, W. Über Sportschäden am Bewegungsapparat. *Med. Klin.*, 1927, **23**, 606–607, 1226–1227, 1305–1307, 1540–1542, 1619–1620.
5. Berthier, A. Étude histologique et expérimentale des Ostéomes musculaires. *Arch. de méd. expér. et d'anat. path.*, 1894, **6**, 601–632.
6. Lexer, E. Das Stadium der bindegewebigen Induration bei Myositis progressiva ossificans. *Arch. f. klin. Chir.*, 1895, **50**, 1–15.
7. de Josselin de Jong, R., and Eykman van der Kemp, P. H. Experimentelle Untersuchungen über die Autotransplantation von Knochengewebe. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1928, **79**, 268–332.
8. Gruber, G. B. Zur Kritik der Callusbildung, Muskel- und Narbenverknöcherung. *Virchows Arch. f. path. Anat.*, 1921, **233**, 401–424.
9. Virchow, R. Über Metaplasie. *Virchows Arch. f. path. Anat.*, 1884, **97**, 410–430.
10. Mallory, F. B. The Principles of Pathologic Histology. W. B. Saunders Company, Philadelphia, 1914, 276.
11. Leriche, R., and Policard, A. Physiologie pathologique chirurgicale. Masson et Cie, Paris, 1930.
12. Sacerdotti, C., and Frattin, G. Über heteroplastische Knochenbildung. *Virchows Arch. f. path. Anat.*, 1902, **168**, 431–443.
13. Liek, E. Experimenteller Beitrag zur Frage der heteroplastischen Knochenbildung. *Arch. f. klin. Chir.*, 1906, **80**, 279–310.
14. Gruber, G. B. Über Histologie und Pathogenese der circumskripten Muskelverknöcherung. G. Fischer, Jena, 1913.
15. Leriche, R., and Policard, A. Les problèmes de la physiologie normale et pathologique de l'os. Masson et Cie, Paris, 1926.

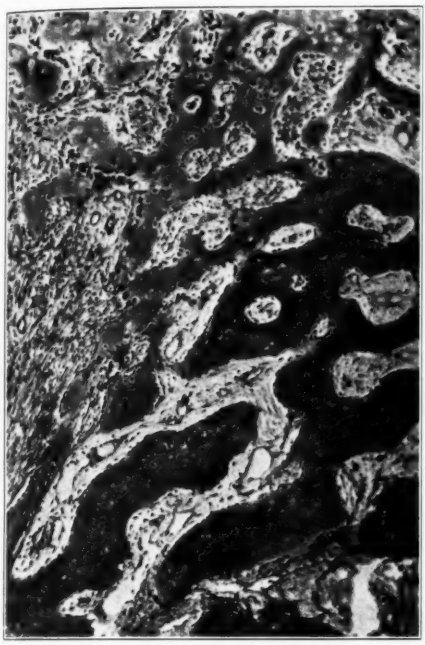
DESCRIPTION OF PLATES

PLATE 123

FIG. 1. Case 1. One of the best differentiated areas showing lamellae of noncalcified osteoid material bordered by osteoblasts. × 50.

FIG. 2. Case 1. The edge of an actively forming osteoid lamella, typical osteoblasts and a mitotic figure. × 800.

FIG. 3. Case 1. An area of active bone formation showing all transition stages from fibroblasts to osteoblasts. × 230.



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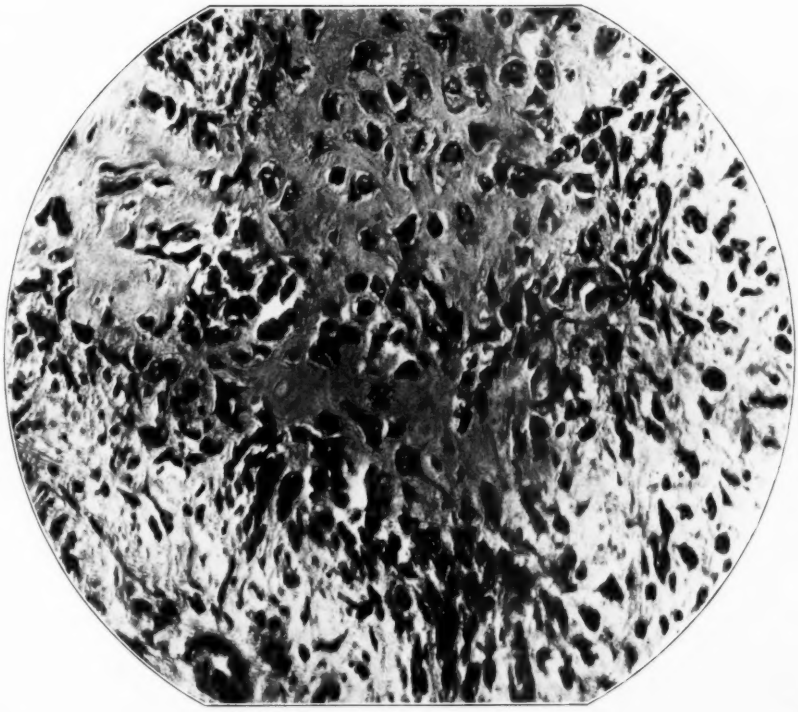


3

Mallory

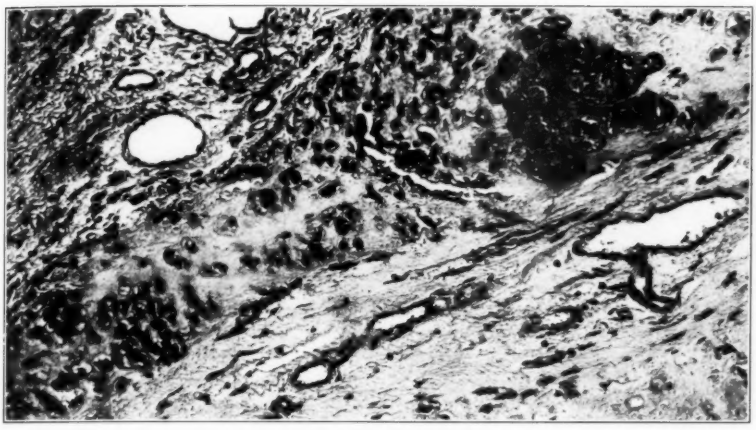
Bony and Cartilaginous Soft Part Tumors

PLATE 124

FIG. 4. Case 3. Active formation of hyaline cartilage at the border of the lesion. Zenker fixation has removed the calcium deposits which, in the formalin-fixed material, almost completely hid the underlying cartilage. × 150.

FIG. 5. Case 5. Sarcoma of the thigh. Giant cells of the foreign body type, hyaline osteoid lamellae, and fibroblastic proliferation. A few large fat cells persist as remnants of the invaded tissues. × 200.

FIG. 6. Case 6. Sarcoma of the breast. Foreign body giant cells, trabeculae of osteoid and rapidly proliferating fibroblasts. × 200.



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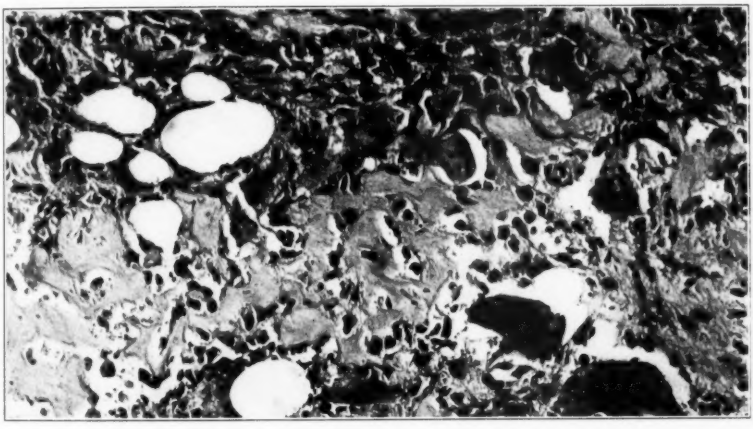

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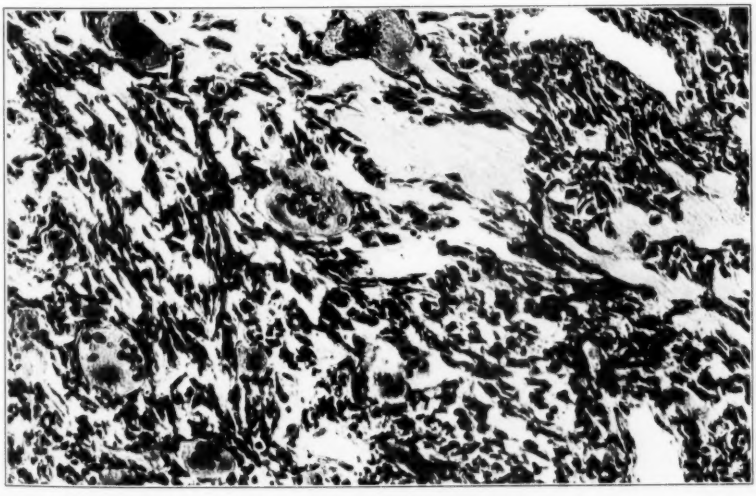

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A CASE OF CONGENITAL LYMPHOBLASTOMA *

CHARLES F. BRANCH, M.D.

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Instances of congenital neoplasms are extremely rare, and this is particularly true of tumors that arise from the hematopoietic system.

In a survey of the literature we found but four such cases mentioned. De Lange [1] reported a case of congenital lymphosarcoma of the mediastinum in an infant who died at 8 weeks of age. At autopsy a mass was found in the posterior mediastinum, measuring 6.5 by 5.5 by 2.5 cm., which had metastasized to the liver. The blood picture was negative. Eichler [2] reported a case of congenital lymphosarcoma of the pancreas in an infant 7 days old. The tumor replaced the entire pancreas and measured 8.7 by 5.5 by 5.5 cm. The other organs were negative and no lymph nodes were involved. The family history was unimportant. Another case of primary lymphosarcoma of the pancreas, which appeared in the literature (L'Huillier [3]), occurred in a 9 day old female infant who died of peritonitis and abscesses of the liver, following phlebitis of the umbilical vein. However, Marxer [4] and other German authorities seem to agree that what L'Huillier thought to be a primary lymphosarcoma was nothing more than a simple hyperplasia of the normal peripancreatic lymphoid tissue which is quite prominent in infants. Chiari,[5] in a personal communication, cited a case of generalized Hodgkin's disease in an infant 2 months old, whose mother had had a tumor mass in the axilla that proved, on biopsy before the child's birth, to be Hodgkin's disease.

REPORT OF CASE †

Clinical History: M. A. C., white, female infant, aged 2 months. The family history was negative. The parents and two siblings were alive and well.

At the eighth week of life the infant began to vomit and a profuse

* Received for publication May 1, 1933.

† I am indebted to Dr. James Vance for permission to report this case.

diarrhea was present. Her condition became rapidly more acute in nature, and the abdomen became distended and tender on palpation. It was decided to operate immediately and an exploratory laparotomy failed to reveal volvulus, intussusception, adhesions, or other evidences of inflammation or malformation. The appendix, as will be noted below, presented a most striking appearance and was removed. The child died early the following day.

Permission was obtained for a limited autopsy and the kidneys, spleen, and portions of the intestines were removed.

Postmortem Examination

Spleen: The spleen weighed 85 gm. and measured 8 by 5 by 2.5 cm. It was enlarged, pale grayish brown, soft, smooth and normal in shape. The cut surface was dark reddish brown, moist, and yielded little pulp on scraping. The trabeculae were poorly defined; the germinal centers were prominent as glistening gray specks averaging 2 mm. in diameter.

Kidneys: The kidneys were similar in appearance. Each weighed 112 gm. and measured 9 by 5 by 4 cm. They were enlarged, still retained much of their fetal lobulation and were firm, smooth, normal in shape, pale gray, and flecked with punctate hemorrhagic areas about 2 mm. in diameter irregularly distributed over the capsular surface. The cut surface showed the normal relation between the pyramids and cortex poorly defined against a pale, grayish brown background. The cortex was 5 mm. in width and the pyramids more widely separated than perhaps is consistent with normal. The capsule stripped with ease, leaving a smooth, coarsely lobulated, glistening surface marked with the punctate hemorrhagic spots mentioned above.

Intestine: Two small pieces were removed. The larger, apparently jejunum, measured 5 by 2 cm. and the wall was 2 mm. in thickness when opened. The smaller piece was similar in appearance and measured 4 by 2 cm. and the wall was 2 mm. thick. The serosa was a pale pinkish gray and was smooth and glistening. The wall apparently was not thickened. The mucosa was not remarkable, except for what appeared to be numerous, minute, submucous, hyperplastic, grayish lymph follicles, having an average diameter of 1 mm.

Appendix: The appendix was 9 cm. long and 1 cm. in diameter. The serosa was smooth, glistening and a chalky pale gray. The or-

gan was firm and less pliable than usual. On section the wall was thickened and edematous in appearance. The lumen, though present, was at no point more than 1 mm. in diameter.

Microscopic Examination

The microscopic appearance of these organs is essentially the same. The normal histological architecture has been materially distorted, if not completely obliterated, by a massive infiltration with cells of the lymphocytic series. The extent of the lesion can best be judged by reference to the photomicrographs. The individual cells of the neoplasm tend to be young lymphocytes or lymphoblasts. The latter present large numbers of mitotic figures. The oxydase reaction is negative. The germinal centers of the spleen and intestine are hyperplastic, while those of the appendix seem to be completely lost in the massive lymphocytic infiltration. The larger vessels show such a preponderance of cells of the lymphocytic series that one is forced to believe that the white count would have been in the hundreds of thousands. It seems highly probable from the evidence at hand that other tissues from this case would have shown essentially the same microscopic appearance as those described.

Discussion

This type of tumor is extremely interesting both from an anatomical and from a pathological standpoint. Clinically the outcome is inevitably fatal. Many interesting academic questions come to mind. Is a picture such as this a true lymphoblastoma or would leukoblastoma be a more appropriate designation? Examination of the other lymphoid organs and the bone marrow would have been of great interest and perhaps would have cleared up many points. Does the disease represent a newgrowth, in the sense that it is truly neoplastic, or is it due to an embryonal imbalance, with a simple overgrowth of one particular tissue — a lymphoid monster, as it were? Do others in the family show any similar lesions, as the mother in Chiari's case did? Were this true, it might suggest a case of true heredity. These four cases are scarcely more than pathological curiosities.

SUMMARY

1. The literature concerning cases of congenital lymphoblastoma has been reviewed.

2. A case of congenital lymphoblastoma has been presented in which the normal histological architecture of the organs has been virtually destroyed by a massive lymphocytic infiltration.

BIBLIOGRAPHY

1. DeLange, C., and Van Goor, W. T. Aangeboren Gezwel vanhet Achterste Mediastinum. *Nederl. Tijdschr. v. Geneesk.*, 1921, **65**, Pt. 2, 828–836.

2. Eichler, P. Ein Falle von kongenitalem Lymphosarkom des Pankreas. *Frankfurt. Ztschr. f. Path.*, 1928, **36**, 326–333.

3. L'Huillier, A. Über einen Fall von kongenitalem Lymphosarkom des Pankreas. *Virchows Arch. f. path. Anat.*, 1904, **178**, 507–509.

4. Marxer, H. Über das Pankreassarkom. *Arch. f. klin. Chir.*, 1925, **135**, 606–627.

5. Chiari, H. Personal communication to Dr. G. K. Mallory.

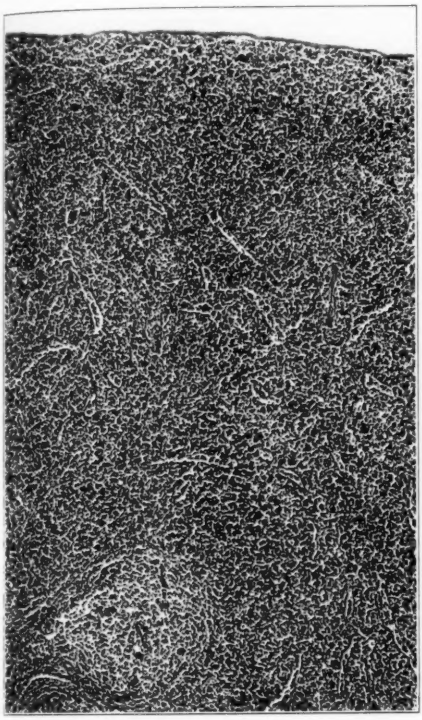
DESCRIPTION OF PLATE

PLATE 125

FIG. 1. Spleen. Note massive infiltration with neoplastic cells, almost total obliteration of the sinusoids, hyperplasia of the germinal centers and extreme thinning of the capsule. × 15.

FIG. 2. Kidney. Marked distortion of the histological architecture and infiltration of the interstitial tissue with neoplastic cells is shown. Many tubules are obliterated. Lymphocytes are found rarely within the tubular lumina. × 75.

FIG. 3. Appendix. Note complete absence of germinal centers and separation of the muscle fibers with uniform infiltration throughout the entire wall. The mucosa is atrophic but intact. × 200.



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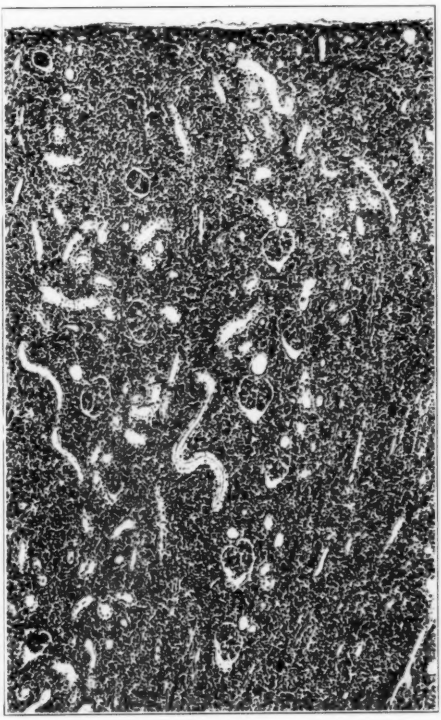

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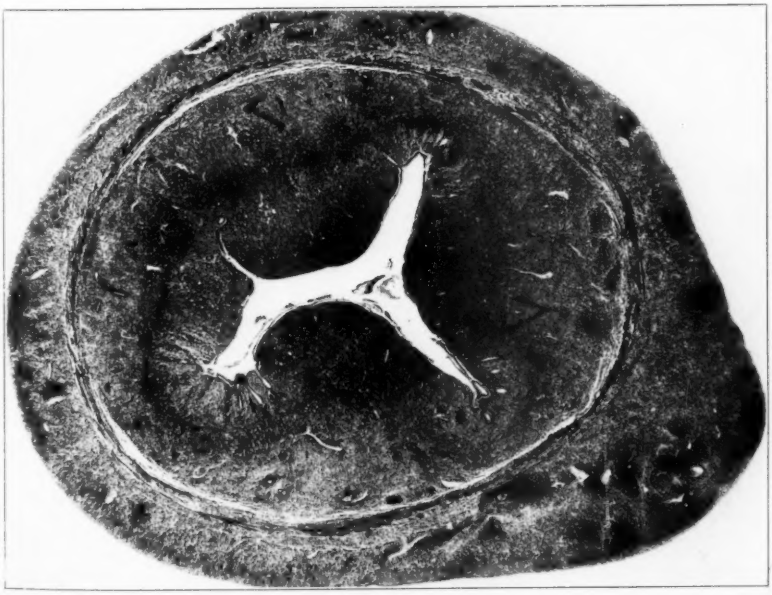

3

Branch

Congenital Lymphoblastoma

THE ANGLE OF THE MITOTIC SPINDLES IN MALIGNANT CELLS *

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The mitotic spindle has received far less attention than other portions of the karyokinetic mechanism, probably due in part to the emphasis placed on the chromosomes and in part to the technical difficulty of satisfactory demonstration of the spindles. A generation ago Hansemann [1] called attention to variations in the mitotic figures, including the achromatic spindles of various types of cells.

TABLE I

(*Ellermann*)

1. GRUPPE			
Megaloblasten		18°	$\sigma = 7{,}2$
Erythrogonien		21°	$\sigma = 6{,}3$
2. GRUPPE			
Lymphoblasten	von Leukämie	42°	$\sigma = 9{,}3$
	von Leukämie	38°	$\sigma = 10{,}3$
	von normalen Organen	39°	$\sigma = 14{,}0$
3. GRUPPE			
Myelozyten	neutrophile	66°	$\sigma = 9{,}0$
	eosinophile	73°	$\sigma = 11{,}0$
Myeloblasten	chronische Leukämie	68°	$\sigma = 14{,}3$
	akute Leukämie	69°	$\sigma = 9{,}7$

Apparently Ellermann [2] was the first to give detailed attention to the variation in mitotic spindles and to use it as a means of differentiating cell types. His investigations centered on the megaloblast and the erythroblast, the lymphoblast, the myelocyte and the myeloblast. At first drawing the mitotic figure and then measuring the angle of the spindle from the drawing, he later proceeded to direct measurement, using a goniometer ocular. He found an error of about 5 per cent in measurement with the goniometer. He discovered striking differences between the mitotic spindles of these various types of cells, which are reproduced in Table I, and his conclusions were that "measurement of mitosis is a method that demands a precise technique and skilled experience but has the great advantage

* Received for publication April 5, 1933.

that through a single observation of the cell it gives a mathematical expression available for cell differentiation instead of an inconstant and difficultly demonstrable variation of nuclear structure." Ellermann based his findings on the measurement of at least 40 mitotic angles for each different type of cell. Several years later Petri [3] used the same methods on a case of myelogenous leukemia and checked closely with the observation of Ellermann.

I have endeavored to apply these same methods to the study of the mitotic angle in various types of malignant cells. Sources of error are numerous and the difficulty of technique is great. None the less there are certain very definite advantages offered by the method. While perhaps not so valuable as Ellermann believes, as an added means of differentiating cells, and particularly cells whose nature is uncertain, it has certain very definite points in its favor. Thus in cells, the nature of which is controversial, such as those of certain tumors of the thyroid, held by some to be carcinomas, by others to be lymphoblastomas, any means of aiding the differentiation of the types of cell involved is worthy of employment.

Our technique has been as follows. Tissues have been freshly fixed in Bouin's, Regaud's, and Zenker's fluids. In general, our results have been fully as good with Zenker fixation as with any other. For stains we have used Mallory's phosphotungstic acid hematoxylin, Masson's modification of Benda's iron hematoxylin, and Mallory's alum hematoxylin and eosin. The distinct staining of the achromatic spindle seems to be more a matter of vagary of fixation than of subsequent handling of the sections. In general, we have been quite successful in demonstration of the spindles.

The angle measured is that at the centrosome, between the two outer linin fibrils of the spindle. Sometimes the outer fibrils are bowed, and here the general course of the spindle is followed, with due regard to the relation of the centrosome and the chromosomes. If the bowing of the spindle is marked, measurement has not been attempted.

Repetition of measurements of the same angle with the goniometer ocular, working at a magnification of approximately 700 diameters, has shown the percentage of error to lie just below 5 per cent, practically the same as that found by Ellermann. A check by the measurement of enlarged photomicrographs of spindles previously measured with the goniometer ocular has shown less than

5 per cent divergence from the mean measurement obtained by the direct method. Thus, it is fair to say that the use of the goniometer is justifiable and it is a great saving in time and expense.

It is important before measuring a spindle to be sure that the two apices of the spindle lie in the same plane, as any rotation will lead to a false widening of the angle, and also that the equatorial plate is in the same axis as the optical axis of the microscope. Moreover, early stages in the separation of the chromosomes must be guarded against, as these will also produce a widening of the angle. The criteria for suitable spindles for measurement may be laid down as follows: the two centrosomes (more in the case of multiple mitoses) must be visible in the same focal plane; the equatorial plate must be seen edgewise, and there should be no lateral shifting as the focus of the microscope is raised or lowered; the separation of the chromosomes must not have begun.

Measurements were carried out on fifteen malignant tumors, of which six were carcinomas, four fibrosarcomas, two osteogenic sarcomas, one melanoma, one glioblastoma, and one Ewing's tumor.* The results obtained in these tumors are shown in Table II. A few scattered measurements, too few to detail, are included in the summarizing table (Table III).

It will be noted that under the fibrosarcomas two sets of readings are given. In this group of tumors we find two distinct types of mitotic figure. In the regions where the cells are elongate, practically all the mitotic spindles are obviously narrow, with angles less than 90° (Fig. 5). The first set of measurements given in the tables represents the narrow angles; the second set, the wide angles. There is a striking spread, more than 30°, between these two types of angle occurring in the same tumor. Certain apparent wide angles prove on careful examination to be due to the existence of double spindles (Fig. 7).

So far as can be determined from the limited number of cases examined, the different tumors tend to run true to type. It is true that in Case 17561, a carcinoma simplex in which only twelve angles, that is, six mitotic figures, were found suitable for measurement, I found a definitely narrow angle, $71° \pm 11.6$, as compared with the other

* Slides used for examination of Ewing's tumor are in the collection of the Registry of Bone Sarcoma, Case UA 32–4, Dr. C. L. Connor, University of California Medical School.

carcinomas; yet even this is a wider mean angle than that found in most of the sarcomas. When we group together all the cases of a given type of tumor we find a difference in the mean values, as may be seen

TABLE II

Results of Measurement of Mitotic Angles

Case No.	Diagnosis	No. of angles measured	Result of measurement	
			Mean	Standard deviation
10753	Epidermoid carcinoma, Grade II	122	92°	±9.7
10526	Carcinoma simplex	40	88°	±5.4
10620	Carcinoma simplex	66	96°	±9.7
17561	Carcinoma simplex	12	71°	±11.6
10519	Carcinoma simplex	56	91°	±2.3
11968	Adenocarcinoma	30	87°	±8.1
117	Glioblastoma	110	61°	±8.4
18204	Melanotic sarcoma	102	56°	±9.6
30–1509	Fibrosarcoma	26 { 14 12	66° 114°	±8.2 ±5.7
32–1917	Fibrosarcoma	24 { 18 6	66° 111°	±13.3 ±6.7
32–2570	Fibrosarcoma	16 { 12 4	70° 101°	±8.2 ±3.8
32–2064	Fibrosarcoma, approaching neurogenic	66 { 20 46	78° 107°	±13.1 ±8.8
R 1362	Osteogenic sarcoma	20	65°	±10.5
33–239	Osteogenic sarcoma	24	73°	±13.5
R 1314	Ewing's tumor	12	88°	±10.7

from Table III. From these values one may conclude that the determination of the mitotic angle is a factor of value in differentiating types of tumors. The difference is particularly clear-cut, provided a fair number of angles are measured, between carcinoma on the one hand and sarcoma on the other. The cell of Ewing's endothelioma,

in the one case studied, has nearly the same angle as the epithelial cell; the spindle is very small as compared with the equatorial plate (Fig. 4).

As stated above, the fibrosarcomas that we have measured thus far show two distinct types of mitosis, the narrow and the wide spindle. As will be noted later, the wide spindle may prove, at least in some instances, to be actually two partly superposed spindles, really a masked multiple mitosis. The osteogenic sarcoma varies distinctly from the fibrosarcoma in exhibiting only one type of mitotic figure,

TABLE III

Values of Mitotic Angles for Different Classes of Tumors *

Diagnosis	No. of angles measured	Result of measurement	
		Mean	Standard deviation
Carcinoma	346	91°	±10.1
Glioblastoma	110	61°	±8.4
Melanotic sarcoma	102	56°	±9.6
Fibrosarcoma	144 (70 / 74)	72° / 108°	±12.6 / ±8.4
Osteogenic sarcoma	44	69°	±12.8
Ewing's tumor	12	88°	±10.7

* Includes measurements not presented in Table II.

a relatively narrow one, the mean being 69° ± 12.8. This is closely approximated by the one case of glioblastoma that we have been able to examine, which showed a mean angle of 61° ± 8.4 (Fig. 3). There is, of course, not a significant difference between these two figures obtained. However, the spindles of this glioblastoma are distinctly rounded off toward the centrioles, in contrast to the very sharp angles of the sarcomas.

The one case of melanotic sarcoma that we have been able to examine showed a comparatively narrow angle, 52° ± 10.3 (Fig. 6). This is the narrowest of the mitotic angles examined, except that of the lymphoblast. I have not yet available a sufficiently large number of mitotic angles to report definitely on the lymphoblast. Pre-

liminary measurements correspond fairly closely with those of Ellermann [2] and of Petri,[3] about 40°, although a few run as high as 50° (Fig. 1).

Another type of tumor of which we have too little material on which to base an opinion is the Ewing's endothelial myeloma. Examination of one case showed a mean angle of 88° ± 10.7. This, however, is based on only twelve determinations. The goniometer

TABLE IV

Readings Obtained in Measurement of Mitotic Angles of Carcinoma Simplex

Case No. 10620

85	86	94	97	90	86
100	105	109	97	102	93
90	91	118	100	90	117
118	110	104	100	116	90
90	90	120	86	98	95
98	97	95	91	90	101
119	112	94	88	93	98
80	87	102	107	93	85
77	83	89	87	96	94
97	99	83	99	90	92
93	104	93	99	96	98

Mean, 96°. Standard deviation, ±9.7.

Case No. 10526

89	85	83	87	99	91
90	87	85	79	88	90
90	101	90	81	98	94
90	90	92	85	95	88
85	87	79	84	85	92
81	82	90	92	86	94
90	83	78	80		

Mean, 88°. Standard deviation, ±5.4.

could not be successfully used in this case because of the small size of the spindle, and enlarged photomicrographs were used.

In order that some idea of the variation encountered in the values obtained for the angles may be given, in addition to the information provided through the standard deviations, I am including Table IV, which gives the direct reading obtained with the ocular goniometer in typical examples of carcinoma simplex, No. 10620, with a fairly high standard deviation, and No. 10526, with a small standard deviation.

One point emphasized by these measurements is the not infrequent occurrence of greater degrees of inequality in the angles of the

two ends of the spindle than can be accounted for by errors in measurement. Whereas in the normal cell apparently a symmetrical spindle is the rule, in the cells of malignant tumors varying degrees of asymmetry are not infrequent. These variations at times are apparent rather than real, as with careful inspection two sets of centrosomes with two overlapping spindles may be distinguished. At times, particularly where angles are apparently very wide, there may be actually two distinct spindles side by side (Fig. 7). In the early stage of the formation of a triaster it is possible to have two more or less closely located centrosomes on one side of the equatorial plate and a single centrosome on the other side. This type of mitosis is always either clearly distinguished or else is hinted at by the irregularity of arrangement of the chromosomes, or a definite branching of the equatorial plate (Fig. 8).

That the angle is to some extent independent of the shape of the cell is well indicated by Figures 1 and 4, in both of which the cells are loosely arranged and similarly shaped, and the angles very different. However, the narrow angles obtained in the epithelial tumor No. 17561 occurred in a closely packed focus of spindle cells, and in spindle-celled portions of sarcomas also the angles tend to be narrow, apparently influenced somewhat by the shape of the cell.

The outer linin fibrils of the spindle usually fail to reach the outer portion of the equatorial plate, as is brought out in all the figures. In certain instances the linin fibrils are bowed outward, as though no stress were exerted on them. No explanation for this has yet been found. In the sarcomas studied the axis of the spindle is parallel to the long axis of the cell, while in carcinomas the axis of the spindle is usually independent of the axis of the cell.

Summary and Conclusions

1. On the basis of the cases thus far studied the angle of the mitotic spindle provides a method by which carcinoma can be distinguished from sarcoma and from lymphoblastoma.

2. Because of the two types of mitosis apparently usually occurring in the same tissue in fibrosarcoma, as distinct from other types of sarcoma, this particular form may be distinguished.

3. The mean values obtained for the mitotic angles in carcinoma are 91°, in melanotic sarcoma 56°, in fibrosarcoma 72° for the narrow

spindles and 108° for the wide spindles, in osteogenic sarcoma 69°, and in Ewing's tumor 88°.

4. Further study of other types of tumor and normal cells is now in progress.

REFERENCES

1. Hansemann, D. Studien über die Spezificität, den Altruismus und die Anaplasie der Zellen. A. Hirschwald, Berlin, 1893.

2. Ellermann, V. Messung der Mitosenwinkel als Methode zur Unterscheidung verschiedener "lymphoide," Zellformen. *Folia haemat.*, 1923, **28**, 207–216.

3. Petri, S. Histologische Untersuchung eines Falles von myeloischer Leukämie mit Messung der Mitosenwinkel. *Folia haemat.*, 1925–26, **32**, 103–114.

DESCRIPTION OF PLATES

PLATE 126

FIG. 1. Case No. 18204. Mitotic spindle in normal lymphoblast from germinal center. Phosphotungstic acid hematoxylin stain. × 2350.

FIG. 2. Case No. A 27–4. Epidermoid carcinoma, Grade 2. Mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. × 2350.

FIG. 3. Case No. 117. Glioblastoma. Mitotic spindle in tumor cell. Hematoxylin and eosin stain. × 1500.

FIG. 4. Case No. UA 32–4. Ewing's tumor (endothelial myeloma). Mitotic spindle in tumor cell. Hematoxylin and eosin stain. × 2350.

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Warren

Angle of Mitotic Spindles in Malignant Cells

PLATE 127

FIG. 5. Case No. 11966. Fibrosarcoma. Mitotic spindle (narrow type) in tumor cell. Phosphotungstic acid hematoxylin stain. × 1500.

FIG. 6. Case No. 18204. Melanotic sarcoma. Mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. × 2350.

FIG. 7. Case No. 32–2064. Fibrosarcoma. Double mitotic spindle in tumor cell. Phosphotungstic acid hematoxylin stain. × 1500.

FIG. 8. Case No. 18204. Melanotic sarcoma. Triaster. Phosphotungstic acid hematoxylin stain. × 2350.

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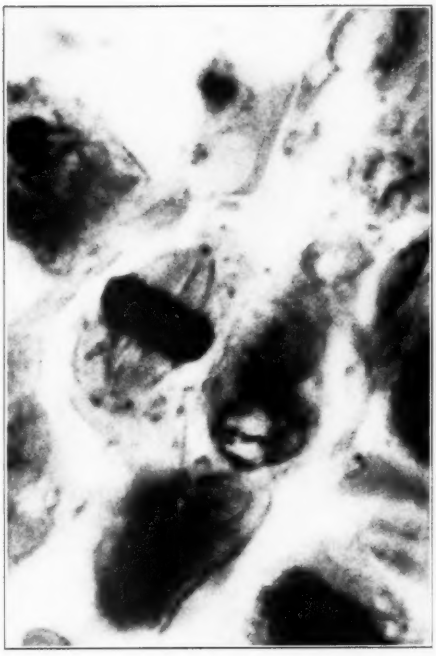

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Warren

Angle of Mitotic Spindles in Malignant Cells

THE EFFECT OF CAESIUM CHLORIDE ON TRANSPLANTED TUMORS OF MICE *

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Numerous experiments have been carried out to determine the effect of various chemical elements on the growth of malignant tumors in animals and in man. Of these elements lead,[1-3] thallium,[4, 5] rare elements such as thorium, yttrium, uranium, and so on,[6-9] and strontium, cadmium and barium,[1c] have received special attention, and valuable information concerning their action on growing neoplasms has been accumulated. As far as is known, however, caesium has been studied only casually in this connection,[7, 11, 12] and the writers have been observing the effect of this element on the growth of transplanted malignant tumors of mice. In the present paper a preliminary report of some of these studies is made.

Caesium is the fifth element in the alkali series and is the most strongly basic of them all, with the exception of element 87, which is not yet available for study. Administered as the chloride, caesium is only moderately toxic for animals. It is excreted quite rapidly, chiefly in the urine but also to a less degree in the feces. Traces of the element, however, may be found in the animal body up to about 9 days after the last injection of the salt.

In the experiments here described caesium chloride, C. P., was the salt uniformly employed. An aqueous solution of this salt containing 25.91 gm. per liter is isotonic with physiological (0.9 per cent) sodium chloride solution, and this concentration was chosen as standard. Subcutaneous injections of small amounts of this solution into mice produced considerable immediate local irritation and the animals were nervous and unusually lively for 5 to 10 minutes after the administration. Previous studies[13] of the physiological effect of caesium chloride on normal mice had shown that 0.2 cc. of the 2.591 per cent solution was the maximum daily dose the mice would tolerate, and 0.1 or 0.2 cc. quantities were usually employed in the experiments. Injections were always made into the subcu-

* Received for publication June 19, 1933.

taneous areolar tissue of the back, well away from the tumor if one were present.

For the experiments the Twort carcinoma was employed. This tumor originated as a carcinoma of the mammary gland of a mouse. It was found to be readily transmissible to other mice if a small fragment of the tumor tissue were implanted subcutaneously. The take rate is somewhat variable but in our experience approximately 80 per cent of the tumors were visibly growing 10 to 20 days after implantation. The tumor is characterized by a tendency to grow quite rapidly, to metastasize to the lungs at a fairly early period, and to undergo early spontaneous necrosis in its central portion. At 8 weeks it reaches moderately large size, averaging about 3 cc. in volume. Metastasis to the lungs has usually occurred at 12 weeks, often earlier.

Histologically the Twort carcinoma is a rather rapidly growing neoplasm obviously of epithelial origin. The tumor cells at the peripheral portions of the growth, where they are viable, are elongated or spindle-shaped, sometimes polyhedral, usually poorly differentiated. They contain large, irregularly oval, often lobulated nuclei in which small numbers of minute, pale-staining chromatin granules are present. The cytoplasm is moderately abundant, faintly acidophilic, and occasionally vacuolated. Large, pale, hyaline nucleoli, from one to five per nucleus, are usually observed. Mitotic figures are abundant. In general the neoplastic cells grow in irregular sheets or cord-like masses without definite gland formation but with a distinct tendency to the production of alveolar structures. The stroma consists of delicate strands of connective tissue in which thin-walled blood vessels are present but not abundant. The major portion of the tumor, beginning in the center, becomes necrotic at an early period and most of the sections show extensive foci of degenerated tumor tissue in which perithelially arranged, small groups of viable cells may often be found. Because the tumor cells are large and rapidly growing the neoplasm is ideal for the study of the effect of an agent that may act primarily on the growing cell.

The white mice used in the experiments were obtained from one dealer and were said to be of the same strain. Nothing is known concerning the racial or hereditary characteristics of the animals, but they were found to be quite healthy and free from disease and

spontaneous tumor development. In general, male mice weighing from 20 to 25 gm. each were employed in all of the experiments.

The technique of implanting the tumor into the mice was as follows. The host mouse was killed with ether and the tumor removed aseptically and placed in a sterile, covered Petri dish. Small blocks of the tissue were preserved in Zenker's fluid or 5 per cent formaldehyde solution for future histological study. All recognizable necrotic material was separated and discarded. From the remaining viable neoplastic tissue small fragments measuring about 0.2 cm. in diameter were prepared. The skin of the mouse to receive the implant was sterilized with alcohol, nicked with sharp scissors, the subcutaneous areolar tissue stretched and a fragment of tumor tissue introduced. The wound edges were drawn together and sealed with collodion and the animal returned to the cage. At first each mouse was anesthetized for the procedure, but subsequent implantation without anesthesia was found to be readily tolerated. Of several hundred mice implanted in this fashion not one developed postoperative infection. Mortality, however, was somewhat higher in the anesthetized mice than in those in which no anesthetic was employed.

Effect of Caesium Chloride, Administered After Tumor Implantation, on the Growth of the Twort Carcinoma

Forty-two healthy white mice, all males from the same breeder's lot, were inoculated on May 6th with Twort carcinoma from a mouse that had received the tumor 86 days previously. After implantation the mice were divided into four groups, one of which was kept as a control. The animals in the other groups were given daily subcutaneous injections of 0.2 cc. of a 2.591 per cent solution of caesium chloride, beginning 1, 10 and 20 days, respectively, after implantation.

At the end of 10 days 8 of the control mice showed small but apparently growing tumors, while 2 had none. The mice of the first group injected with caesium chloride solution had suffered high mortality, 8 of the mice having died, apparently from an epizoötic. Of the 2 that remained 1 had a growing tumor, the other had none.

In the other two groups the tumor take rate was approximately that of the control series: 9 alive in one group — 8 with small tumors

and 1 with none; 8 alive in the other — 6 with small tumors and 2 with none.

At this time daily caesium chloride injections were begun in the group in which 9 mice were alive. At the end of 10 days 5 were living, all with definite tumors that continued to grow in spite of continued caesium chloride injections.

Twenty days after implantation the last group, now reduced to 6 animals, 4 of which had definite tumors while 2 had none, were started on daily doses of caesium chloride. At the end of a month of treatment one of the four tumors had regressed but the others had grown considerably. The 2 mice without tumors continued to be tumor-free.

The mice of all groups except the controls continued to receive daily injections of the caesium chloride solution. The tumors grew progressively and one by one the animals died, those that had received caesium as well as the controls. Once the tumors appeared caesium chloride had no evident effect, except in a single case where an apparently growing neoplasm regressed after caesium chloride injections were started. It cannot be said that in this case the regression was due definitely to the effect of caesium, since spontaneous regressions are known to occur.

Effect of Caesium Chloride on the Large, Growing Twort Carcinoma

Since caesium chloride seemed to have no great effect upon the growth of the Twort carcinoma when injections were begun soon after implantation, it was decided to study the effect of the salt upon large, well developed tumors. A group of mice had been implanted with tumors on February 10th for another experiment. By April 26th approximately 80 per cent of the control animals in this series had large tumors. Twenty of these mice with well developed tumor masses were divided into two equal groups, one of which was kept as a control.

Daily injections of 0.2 cc. of the caesium chloride solution were begun on the second group, with the result that none of the injected animals became tumor-free. In several cases, however, the neoplastic tissue seemed to be softer and more extensively degenerated

in these animals than in the controls. However, death, either from the neoplasm itself or from some associated secondary cause, occurred in all cases, the first mouse dying on May 14th, the last on July 22nd. The control mice likewise showed no tendency to tumor regression and death occurred on various days between May 12th and July 2nd.

Careful gross examination of the tumors from the animals that had received caesium chloride showed large, soft, necrotic, cystic masses, the centers of which were broken down and liquefied. As usual the only viable tissue, if present, was found at the periphery of the nodules. In some cases little if any living tissue could be detected grossly. Metastases, however, were generally present in the lungs, so that caesium chloride injections had had no deterrent effect upon the formation and growth of metastases.

The tumors from the control mice were similar in most respects to those of the treated animals, except that necrosis appeared less generalized and viable tissue was uniformly found. Pulmonary metastases were present in practically every animal.

Histologically the neoplastic tissue from both experimental and control animals differed but little, except for the fact that, as was noted grossly, necrosis of the tumors was sometimes more extensive in the treated animals. But whether or not this effect was due to caesium it is impossible to say. The caesium salt was thus found to have no perceptible effect on the size of the growing Twort carcinoma, and doubtful, if any, effect upon its gross and histological structure.

Effect of Preliminary Injections of Caesium Chloride on the Take Rate and Growth of the Twort Carcinoma

Forty healthy, male, white mice from the same breeder's lot were divided into two equal batches which were caged separately. To one lot daily subcutaneous injections of 0.2 cc. of the standard caesium chloride solution were given for a period of 9 days. For some unexplained reason, possibly caesium poisoning, 8 of the treated mice died during this time. On the 10th day the living experimental animals and 12 of the controls were implanted with the Twort carcinoma which came from a mouse that had received the tumor 63 days previously.

The experimental mice were then continued on daily injections of caesium chloride, a smaller amount, 0.1 cc., being employed. On the 18th day 5 of the treated mice had definite tumors (41.6 per cent) and 7 (58.4 per cent) had none, while 10 of the controls (83.3 per cent) had growing tumors and only 2 (16.7 per cent) had none. On the 36th day the findings in the control group were the same. Of the 5 treated mice with tumors, however, 2 had become practically tumor-free.

The tumors in the control mice were large, elastic and rather soft to the touch. On removal from the body they were smooth-surfaced, slightly lobulated and friable. On section the cut surfaces of the viable portions were pinkish with a pearly luster, and the entire mass resembled the typical Twort carcinoma described above, with soft, necrotic, cystic centers. There was considerable evidence of invasion of the abdominal wall, but no peritoneal or pulmonary metastases were found at this stage of growth.

Histologically the tumors were typical carcinomas of the type described above. Foci of necrosis were everywhere visible. In the viable portions the tumor cells were large, undifferentiated, and rapidly growing. Mitotic figures were abundant. A rough average of eight to nine per field was determined by counting at random a total of thirty oil immersion fields in sections from 6 of the animals.

The tumors in the treated mice were smaller, firmer, and more solid than were those in the controls. Grossly they were smooth-surfaced, finely nodular, pinkish, and friable, although less friable than the tumors in the control animals. On section, however, there was little to differentiate these tumors from those of the untreated mice. Viable tissue was peripherally placed and central necrosis of the mass was no more marked than in the animals described above. Metastases to the lungs and extension into the peritoneal cavity were not present.

From a histological standpoint the tumors resembled those of the untreated controls, consisting of a peripheral zone of moderately large, undifferentiated, polyhedral or elongated epithelial cells growing in alveolar masses and surrounded by delicate, slightly vascular, fibrous stroma. Rarely there was a tendency to gland formation. Widespread foci of necrosis were present, but neither the extent nor the character of the necrosis differed from that noted above. The cytoplasm of the tumor cells was somewhat more basophilic than

that of the control tumors, but it was not appreciably diminished in amount. Cell nuclei likewise showed no morphological differences. Nucleoli were present and mitotic figures were relatively abundant. In thirty oil immersion fields taken at random an average of seven to eight mitoses per field was found. Thus in spite of gross differences between the tumors of control and treated animals no histological differences of note could be detected.

Sections taken from the subcutaneous tissues of the animals, both experimental and control, in which tumors did not appear, showed little of note histologically. At 10 days necrotic tumor implants, surrounded by an inflammatory reaction, were found. At later periods no tumor tissue at all could be discovered.

A second lot of 34 male white mice was divided into two groups, to one of which daily subcutaneous injections of 0.1 cc. of a 2.591 per cent solution of caesium chloride were given for 4 days, after which the dose was increased to 0.2 cc. for 9 days. The Twort carcinoma was then implanted into all the mice, both experimental and control. After implantation daily injections of 0.2 cc. caesium chloride were given to the experimental mice.

At the end of 36 days 4 of the experimental mice were dead. Of the living animals 6 (46.2 per cent) had no tumors, while in 7 (53.8 per cent) small ones were present. Of the control mice 4 had died. Eleven (84.6 per cent) of the survivors had large tumors while 2 (15.4 per cent) had none.

The size of the tumors in the treated and untreated groups was significant. The average diameter of the tumors in the injected mice was 0.9 cm.; the total volume of tumor tissue in all the animals together was 3.3 cc. The average diameter of the tumors in the control animals was 1.25 cm.; the total volume of all tumor tissue was 14.7 cc. There was thus a striking difference between the actual amount of tumor tissue that grew in the two sets of animals.

Grossly the tumors from the control mice were fairly large, a few measuring 2 to 2.5 cm. in diameter. They were soft, friable, lobulated, necrotic and often superficially ulcerated. Their centers were liquefied. Histologically the viable portions consisted of rapidly growing cells similar to those described above. An average mitotic figure count of seven to eight per field was determined from a study of thirty oil immersion fields. Considerable necrotic tissue was present.

The tumors of the injected animals were much smaller than those of the controls. They were firmer to the touch and seemed more solid but on section their centers, like those of the control tumors, were broken down and liquefied. Histologically there was little to differentiate the two types of tumors, treated and untreated. The neoplastic cells of the treated tumors appeared more densely packed together and were slightly more basophilic than those of the controls. In thirty oil immersion fields an average of seven to eight mitoses per field was found. Active cell division was thus as abundant in the treated tumors as in the untreated.

Caesium chloride injections, begun 9 to 13 days before tumor implantation and continued thereafter, apparently diminish the take rate of the Twort carcinoma and result in the growth of tumors which are grossly considerably smaller and more compact than those of control animals, but which show practically no histological differences when the tumor tissues are studied by the usual tinctorial methods. There is little evidence that the tumor, once actively growing in the animal body, is perceptibly affected by the administration of the caesium salt.

Discussion

In considering the activity of the cell nucleus in rapidly growing normal or neoplastic tissues the question arose as to the effect a strongly basic ion might exert on nuclear activity, if it were introduced into the reaction system. One may conceive of the cell nucleus as containing, among other substances, a readily oxidizable sugar, phosphoric acid, certain nitrogenous compounds, and various basic ions, sodium, potassium, calcium, and so on. Some of these substances, according to Levene and Bass,[14] are capable of combining to form the compound that is peculiar to every cell nucleus, namely, nucleic acid. This complex substance, in its simplest form, Levene believes to be "an ester of phosphoric acid and an organic radicle, the latter consisting of a sugar and a nitrogenous component which is a cyclic derivative of urea." In the opinion of this investigator animal nucleic acid (zoönucleic acid) is a tetranucleotide consisting of four molecules of phosphoric acid attached to four molecules of a reduced pentose, namely *d*-ribodesose, to which in turn are linked four nitrogenous compounds, adenine, guanine, cytosine and thymine.

In any given cell nucleus the general proportions of the constituents of nucleic acid, in solution and ready to combine, are probably fairly constant. So, too, are the various basic ions. The product of the reaction system, phosphoric acid-pentose-basic ion, is therefore pentose-phosphoric acid ester and basic phosphate, presumably in a fairly constant ratio. However, if one or the other of the various reacting substances were to be altered or replaced, there should theoretically be some effect upon the activity of the system. If, for example, the unstable pentose, *d*-ribodesose, were to be increased, we might well conceive of possible resultant increased activity of the cell nucleus because more nucleic acid, so readily capable of hydrolysis and oxidation, would tend to be formed.

If, on the other hand, a powerful basic ion were to be introduced into the system we might conceive then of the formation of increased amounts of basic phosphate at the expense of the nucleic acid. This would theoretically result in the formation of a relatively inert substance at the expense of an unstable compound capable of marked chemical activity. It is not unreasonable to conclude, therefore, that a nucleus containing such a powerful basic ion would be less active than usual. If such a condition could be produced in rapidly growing malignant cells the result might be interesting.

To test the validity of such an hypothesis the experiments outlined above were devised and the element caesium, the most strongly alkaline and electropositive of all of the available alkali elements, was chosen as the actively basic ion to be introduced into the reaction system. So far the results have been disappointing. Caesium chloride, the salt of caesium that was uniformly employed, injected soon after the implantation of the Twort carcinoma into mice, has no appreciable effect upon the growth of the tumor. If daily injections be started after the tumor has reached a relatively large size the salt has no effect whatever, except possibly that of increasing the amount of necrosis occurring in the tumor, yet even this is uncertain.

If, however, the tumor be implanted into mice already saturated with caesium, and which continue to receive daily injections of the caesium salt, there is evidence of some deterrent effect of the element upon the growth of the neoplasm. In the first place, the percentage of takes is less in the caesium-injected mice than in the controls, 41.6 per cent in one series and 53.8 per cent in another.

The control mice in these series showed a much higher percentage of takes, 83.3 per cent and 84.6 per cent respectively. It is thus possible that the tumor fragment, introduced into a tissue medium in which highly alkaline ions are supposedly present, is unable to establish itself readily as a growing implant. In the second place, the individual tumors, if they do appear in the treated animals, are smaller and somewhat more firm in consistence than the control growths. However, they exhibit practically no tendency to regress once they have developed, and histologically they show no readily recognizable structural changes that would differentiate them from the controls.

Caesium, if present in the animal body before tumor implantation, thus exhibits an apparent deterrent effect upon the take rate and the subsequent growth of the Twort carcinoma, as indicated by the clinical character of the growth. Morphologically, however, no significant changes can be found in the tumor cells themselves. The probability is that the effect produced by caesium is essentially chemical in character, with no recognizable alteration of the cellular structure. From careful cytological study of the transplanted tumor tissue in this small series of caesium-injected animals it is impossible to discover any obvious diminished proliferative activity on the part of the tumor cells. These cells are apparently as abundant, as healthy and as active in the tumors of treated mice as they are in the neoplasms of the control animals.

Summary and Conclusions

The theoretical hypothesis that the introduction of strongly basic ions into the nuclear complex might greatly reduce nuclear activity is not supported by the study of the effect of caesium upon the growth of the Twort carcinoma of mice. There is no evidence in these experiments that the presence of the element caesium in the body of a mouse with established Twort carcinoma affects in any way the capacity of the tumor cells to divide and multiply. There is, however, suggestive evidence to indicate that necrosis of the neoplastic tissue may be more extensive in the caesium-treated animals. That caesium under certain conditions has a deterrent effect on tumor growth is indicated, and further studies of this property of the element are in progress.

REFERENCES

1. Levi, A., and Volta, E. L'azione del piombo e dello zinco sui tumori sperimentali dei topolini bianchi. *Arch. di biol.*, 1930, **7**, 25–33.
2. Bell, W. B. Present position of lead therapy in malignant disease. *Brit. M. J.*, 1929, **1**, 431–437.
3. Wood, F. C. Experimental investigation of lead therapy. *Am. J. Roentgenol.*, 1930, **23**, 299–303.
4. Engman, M. F., Jr. A study of the effect of thallium acetate upon the growth of the Flexner-Jobling tumor in albino rats. *Am. J. Cancer*, 1932, **16**, 847–853.
5. Lansbury, John. Studies on the action of thallium on the Flexner-Jobling carcinoma transplanted into the white rat. *Proc. Staff Meetings, Mayo Clinic*, 1932, **7**, 529–534.
6. Maxwell, L. C., and Bischoff, F. Studies in cancer chemotherapy. X. The effect of thorium, cerium, erbium, yttrium, didymium, praseodymium, manganese, and lead upon transplantable rat tumors. *J. Pharmacol. & Exper. Therap.*, 1931, **43**, 61–70.
7. Simpson, B. T., and Marsh, M. C. Therapy of spontaneous mouse cancer. *Ann. Surg.*, 1931, **93**, 169–179.
8. Pack, G. T., and Stewart, F. W. Uranium-thorium colloid in the treatment of carcinoma. *J. Cancer Research*, 1930, **14**, 152–165.
9. Hocking, F. D. M. The employment of uranium in the treatment of malignant new growths. *Rep. Internat. Conf. Cancer*, London, 1928, 253–255.
10. Maisin, J. Essai de métallothérapie spécifique des cancers humains. *Compt. rend. Soc. de biol.*, 1931, **108**, 808–810.
11. Ishiwara, F. Ueber den Einfluss 58 verschiedener chemischer Verbindungen auf den Tierkrebs. *Gann*, 1927, **21**, 1–5.
12. Roffo, A. H., and Calcagno, O. Étude biologique de l'action des vanadates de sodium, de potassium, d'ammonium, de lithium, de rubidium, de caesium, de magnésium, et de calcium sur le développement des tissus normaux et néoplastiques in vitro. *Ann. de physiol.*, 1931, **7**, 649–720.
13. Graham, C. F., and Wright, A. W. The effect of caesium chloride upon the albino mouse. (In press.)
14. Levene, P. A., and Bass, L. W. Nucleic acids. *Am. Chem. Soc. Monograph Series, No. 56*, Chemical Catalogue Co., New York, 1931.

THE DIAGNOSIS OF TUMORS BY ASPIRATION *

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During the past three years the author has studied smears of material from some 2500 tumors obtained by aspiration with an 18 gauge needle. The method of securing the tissue has been described by Martin and Ellis,[1] but since the actual technique is of considerable importance it may be described again in brief. The needle used is an ordinary 18 gauge needle attached to a Record syringe. The skin is infiltrated by a drop of novocaine and a very small puncture wound is made with a bistoury to avoid contamination of the tissue to be aspirated with surface epithelium. The needle is introduced with the piston *closed* until it is felt to enter the neoplasm. Then the piston is partially withdrawn, thus creating a negative pressure within the syringe, and the needle is advanced into the tumor. Portions of tumor are forced by the negative pressure into the needle. The manoeuver is usually repeated once without withdrawing the needle from the mass. When the needle is withdrawn care is taken to release the piston gradually, in order to prevent a sudden spraying of the contents of the needle over the walls of the syringe. Material obtained is placed on a slide and smeared out by pressure with another slide. Gentle heat is applied and the smear is dropped into alcohol for a minute or two, after which it is stained with hematoxylin and eosin, dehydrated, cleared and mounted like any section. If much fluid is obtained small fragments of tissue are selected with a wire loop and smeared on slides, or, if sufficiently large, they are sometimes sectioned. Blood clots may be sectioned in the same manner as tissue.

It is the purpose of this paper to discuss the usefulness of this method in diagnosing tumors and to call attention to certain pitfalls and limitations. In the Memorial Hospital laboratory over 6000 pathological specimens are received annually for diagnosis. Many of these are small biopsies. It is not the practice of the clinicians to use the method of aspiration when the ordinary biopsy can be obtained from an ulcerating surface, but under many circumstances

* Received for publication April 22, 1933.

where pathological diagnosis is required it is impossible or undesirable to attempt the usual form of biopsy. Furthermore, certain therapeutic procedures employed in this hospital make it essential, or at least simpler for the patient, to depend frequently on aspiration for diagnosis when others would secure a surgical biopsy.

Material obtained by aspiration must necessarily consist of the more unattached constituents of a tissue, cells that are readily displaced into the needle by a little manipulation. Hence, most smears of such tissues consist of loosely arranged sheets of cells or, in the more cellular tumors, of showers of isolated cells or clusters of a few elements. Since the smears are subjected to but little fixation the tumor cells appear as a rule larger than in fixed tissues. For the most part the coarser morphology of the tumor is lacking, the relations of parenchyma to stroma often cannot be determined and in interpreting the smear one must relearn by experience the characteristics of the various tumors. To do this it will aid greatly if one aspirates gross tumors coming to the laboratory for the usual type of diagnosis, thus confirming by sections the pictures shown in smears. Before interpreting an aspiration it is the writer's custom to be familiar with the clinical history of the case and the exact location of the suspected mass, together with the clinician's impression of the diagnosis. The pathologist should know from the clinical description what types of tumors may present themselves in a given clinical setting. Only then is it possible to put together the various minutiae of a smear and render the probable diagnosis. It may be well to recite briefly a case in point.

The patient was a middle-aged woman who entered the clinic of the Memorial Hospital complaining of a recurrent subcutaneous mass in the scalp. She presented a hard, lobulated, movable, subcutaneous bulging tumor over the vertex. No source of metastasis was found. Aspiration yielded pure cartilage (Fig. 1). The obvious diagnosis was chondroma, yet the location of the tumor was scarcely consistent with that diagnosis; the tumor was movable on the deeper structures and radiographs of the skull revealed no bone defect. It was therefore necessary to consider in the diagnosis some type of tumor that could occur in the scalp without relation to bone, and that could yield cartilage on aspiration. The most obvious suggestion was, of course, a tumor of cutaneous glands — either sweat glands or sebaceous glands — with extensive cartilaginous metaplasia, such as occurs in salivary gland tumors. Later examination of the tumor after surgical removal proved it to be an adenocarcinoma of the sweat glands with marked cartilaginous metaplasia, thus confirming a diagnosis rendered upon a combination of data derived from aspiration and from clinical experience. Neither alone could have sufficed.

Under most circumstances it has not been necessary for us to determine from aspirated material the exact histological subvariety of tumor. The usual question involved is merely the presence or absence of a malignant tumor, since the clinician, when informed that a lesion contains cancer cells, is usually quite capable of determining from other data the essential type of disease. Merely ascertaining the presence of cancer is much simpler than determining its particular variety. The latter is oftentimes impossible from smears.

Conservative surgeons or radiologists may be appalled at the idea of introducing a needle through sound tissue into a cancerous mass. The avoidance of trauma to a malignant tumor is doubtless highly desirable, yet quite impossible if one is to secure a histological diagnosis, and the burden of proof that surgical excision is a less traumatizing procedure than simple needle aspiration appears to the writer to rest on those who advocate the former method. Suffice it to state that at the Memorial Hospital no untoward result can as yet be attributed to needling a tumor, whereas unfavorable consequences have been observed to follow surgical biopsies, particularly when performed by surgeons unfamiliar with the hazards of biopsy in certain types of tumors, especially those of bone.

It was emphasized at the beginning of this paper that biopsy by aspiration is not performed in ulcerating lesions where a small bit of tissue is readily secured with the biopsy forceps. In any large tumor clinic, however, it is necessary for various reasons to secure specimens from deep seated lesions where an ulcerated surface is not accessible.

The remainder of this brief paper is best devoted to a discussion of various types of tumors where aspiration is of practical value. Prominent among such lesions is the possibly cancerous cervical node. In many surgical clinics a routine neck dissection is performed on all patients with intra-oral carcinoma when the disease is still operable. At the Memorial Hospital the tendency in recent years has been to limit more and more this rather drastic procedure. At the present time very few neck dissections are done. Since for the most part intra-oral carcinomas metastasize to but one or two nodes, usually situated at the carotid bulb, it is the practice to radiate the neck externally and, later on, in the case of the more radioresistant lesions, to expose the nodes surgically and to implant an adequate amount of gold radon seeds in persistent node metastases. The ultimate out-

come of this procedure, as judged by five year statistics, is still in doubt and it may be abandoned or greatly modified. Since, however, the results of surgical treatment of established metastatic carcinoma of the cervical lymph nodes are rather unfavorable, and since many patients present an inoperable or doubtfully operable setting, an experiment with a more conservative method of therapy is fully justified. Moreover, in the case of many types of intra-oral carcinoma — notably the transitional cell carcinomas and lymphoepitheliomas of the tonsil, base of the tongue, soft palate, epiglottis, arytenoid cartilages and pyriform sinuses — the metastatic foci are often far beyond the hope of even palliation by neck dissection. Since for statistical purposes, as well as for determining the immediate therapeutic procedure, it is desirable to have histological proof that an enlarged cervical node contains metastatic tumor, every suspected node is subjected to aspiration. We have examined 725 such aspirations and find it for the most part simple to distinguish cancer cells. The large sheets of flattened, spindle or polyhedral cells, with central masses of pink-staining cells more squamous in shape, stand out in sharp relief from the typical lymphocytes of the node. The cells of transitional cell carcinoma are smaller, usually more spindle in contour and lack squamous characteristics, unless the node has been subjected to radiation. Oftentimes a metastatic node undergoes central liquefaction and under these circumstances only masses of débris and unattached pink-staining, rather hyaline squamous cells may appear in the smear. Calcification may occur. Reaction to necrotic squamous pearls leads to the appearance of foreign body giant cells, which may give rise to confusion with tuberculosis. The history of the case is of decisive significance in this diagnosis. Much this same appearance may arise in smears from the contents of a branchiogenetic cyst. In fact the cyst, with its sloughing epithelium, cellular detritus, calcified material and lymphocytes may duplicate the necrotic metastasis of squamous carcinoma. Again, the history of a prior intra-oral carcinoma makes the diagnosis. When no primary tumor is found repeated aspiration may be necessary for diagnosis. Sooner or later, clusters of typical tumor cells will appear in fluid aspirated from the metastatic deposit, provided the extent of collapse of the cyst or its rate of refilling does not clear up the diagnosis before repeated aspiration is necessary.

Aspiration finds another field of usefulness in the diagnosis of

lesions of the antrum. In their earlier stages, when they present no ulcerating surface either in the nose or palate and when radiographs show no bone destruction permitting radiographic diagnosis, the aspirating needle is regularly used to distinguish antral carcinoma from either empyema or polyposis. Our records contain data on 50 aspirations of antral lesions. In some cases it has been impossible to return any diagnosis other than carcinoma. In many instances it has been possible to distinguish by their finer characteristics such specific types as epidermoid carcinoma, Schneiderian carcinoma by its mixture of mucus-containing cells, salivary gland tumors by their small cells, cystic adenoid features, and mixture of mucoid, spindle cell areas. In one instance it was possible to differentiate carcinoma from osteogenic sarcoma, thus relieving a patient from the distressing consequences of a surgical and radiological assault designed for the cure of carcinoma of the antrum. In the case of this patient a surgical biopsy would have subjected an osteogenic sarcoma to the well known consequences of incision through an infected field, whereas the aspiration was tolerated perfectly.

The aspiration of centrally located, destructive lesions of the jaw bones is often useful. The radiologist is at times at a loss to distinguish the simple cyst from either adamantinoma or giant cell tumor. The clinician at times hesitates between the diagnosis of extension of oral cancer to the bone or osteomyelitis. Our laboratory contains records of 57 aspirations of the jaw bones, mostly of the mandible. The simple cyst yields as a rule a considerable quantity of straw-colored fluid and on centrifuging one usually recovers scattered, flat, pink-staining polyhedral cells, leukocytes and crystalline material. The giant cell tumor is readily recognized by the large numbers of typical epulis giant cells. Adamantinoma yields in its typical form bits of spindle cell tissue surrounded by columnar basal cells. Very few adamantinomas have occurred since aspiration was instituted and we cannot say that their different degrees of aggressiveness can be recognized from smears. The cystic adamantinoma may present a wall lined by tissue resembling the *débris paradentaire* of Malassez. It is essentially an adamantinoma, yet it may lack features that distinguish it in smears from more aggressive tumors of similar origin.

The parotid tumors are peculiarly suited to aspiration. At the Memorial Hospital it is the practice to excise the freely movable

mixed tumor and to depend on radiation in various forms for the treatment of the inoperable mixed tumor or the various types of true malignant carcinomas. Smears of mixed tumors (Fig. 2) show various combinations of cartilage, mucinous tissue and epithelium. Because these features are absent and the smear shows epithelium only, it does not necessarily mean that the tumor is a fully malignant carcinoma capable of metastasis, since the needle may reach but one portion of a tumor of quite diverse structure. The clinical features of the case are of great importance when the characteristics of the smear lead to doubt. In most instances no difficulty is encountered in recognizing the highly malignant tumor from smears (Fig. 3). We have studied 66 parotid aspirations.

When the primary lymphadenopathies, Hodgkin's disease, lymphosarcoma, pseudoleukemic lymphoma, Brill-Symmer's lymphadenoma and the like, are suspected from clinical examination, an aspiration is frequently performed to rule out the presence of metastatic tumor. With metastatic tumor eliminated it is preferred to submit the patient to a surgical biopsy rather than to depend on aspiration. It has been possible to make a tentative diagnosis of Hodgkin's disease from smears when the tissue showed a distinct eosinophilia or many Sternberg cells, yet for the most part it is our feeling that the fixed smear is unreliable. It will be remembered that Forkner [2] reported little difficulty in distinguishing various types of primary lymph node lesions by examining supravitally stained preparations of bits of tissue obtained by inserting a dental broach through a trocar introduced into the node. We have not examined supravital preparations. We find oftentimes great difficulty in distinguishing or classifying primary lymph node lesions when large, excellently stained specimens are available for study. Hence, we are sceptical about the possibility of making such diagnoses from smears. Certain highly characteristic lymphadenopathies, such as Gaucher's disease, may be readily recognized. Spleens from cases of suspected Gaucher's disease have been aspirated on two occasions and the diagnosis subsequently confirmed by splenectomy.

Nearly 500 breast lesions have been subjected to aspiration. The indications for aspirating a breast tumor may be tabulated as follows:

1. For confirmation of diagnosis in patients who, because of something in their general physical status, are to be selected for treatment

by irradiation alone, and from whom it is not possible to secure tissue for examination unless the patient comes to autopsy.

2. For confirmation of diagnosis in young patients, or when tumors develop in the course of lactation or pregnancy, under which circumstances certain clinicians feel that radical surgery is not the method of choice in treatment on account of the usually unfavorable outcome of the case.

3. To rule out suspicious nodules in the opposite breast in patients presenting an otherwise operable setting.

4. To differentiate the rapidly growing fibro-adenoma from circumscribed mammary carcinoma.

5. To distinguish between recurrent mammary carcinoma and traumatic fat necrosis.

6. To differentiate the thick-walled, deep-seated abscess or cyst from carcinoma. To distinguish diffuse suppurative mastitis from carcinoma accompanied by marked inflammation.

7. To diagnose the deep-seated carcinomatous nodule in fat breasts where palpation is difficult.

Whereas these suggestions offer many problems often settled by the use of an aspirating needle, the procedure is so simple and useful that at the present time almost every breast lesion that presents itself in the breast clinic is aspirated if the least possibility of mammary carcinoma is considered. It requires some degree of skill to obtain cells from a deep-seated tumor, or especially from the sclerosing fibrocarcinomas. If the clinician suspects carcinoma and receives a negative report from his first attempt at aspiration, the report should not deter him from repeating the procedure.

The recognition of breast carcinoma in the smear is relatively simple. Carcinoma cells are large, rather loose and hyperchromatic. They are irregular but tend to appear in loose sheets. Alveolar arrangements are rarely seen. When the tumor cells are small, as they are in the more malignant forms of mammary carcinoma, the chief points in their recognition are their looseness and their irregularity. The cells of a fibro-adenoma may be as large as those of mammary carcinoma; those of proliferated ducts may be as large as the smaller cell types of mammary carcinoma, yet both maintain a coherence (Fig. 4) not found in carcinoma cells (Fig. 5). The spindle cell stroma of some fibro-adenomas permits

their ready detection. Search for mitotic figures has not proved of practical assistance in furthering diagnosis. Other features of the material far outweigh in importance the mitotic figure.

There are borderline pictures that one had best refrain from interpreting. The markedly atypical proliferating and desquamating cells of pronounced Schimmelbusch-Reclus disease may duplicate in smears all the features of mammary carcinoma. The author has once confused these two diseases. The cells of the ducts in ordinary male gynecomastia may offer difficulties. In several cases aspiration of male breasts yielded cells much larger than one would have anticipated from the structure of the normal male breast, yet the cells were uniform in size and definitely coherent. It might be suspected that the lactating breast would be a source of confusion. So far we have had little experience in this field but on two occasions the presence of fat-filled cells and sheets exhibiting an alveolar structure with a double epithelial layer rendered a diagnosis differential from carcinoma possible. It must not be inferred that diagnosis is always simple and that no errors have been made. Until the pathologist has familiarized himself with the various pitfalls errors are certain to occur. They can be corrected only by the knowledge gained from experience. So far, however, the advantages of the method have far outweighed in importance the exceptional error.

There have been 41 aspirations of suspected lung tumors. Aspiration has been used to distinguish between primary carcinoma of the lung and various septic processes, when the lesions are so situated that they are not accessible to the bronchoscope. The recognition of primary bronchogenic carcinoma is simple, but no attempt has been made to distinguish different types, such as epidermoid versus primary adenocarcinoma of mucous glands. The differentiation of primary from metastatic lung tumors has not as yet been required, since radiographs are undoubtedly more decisive in this regard than a smear. In one instance a needle aspiration enabled us to distinguish a recurrent tumor of the pleura, secondary to a peculiar teratoid mixed tumor of the breast, from aneurysm. In another it was possible to detect the daughter cysts of echinococcus in fluid from a dense rounded "tumor" in a patient on whom serological tests for echinococcus had been negative. Save in the case of the last patient, who had a considerable immediate reaction following the evacuation of a rather large amount of fluid, no untoward results have followed

puncturing the lung. In several cases repeated punctures have been required for diagnosis.

The interpretation of smears of pleural or peritoneal exudates contains numerous elements of danger. It is our feeling that such smears are highly unreliable. The difficulty comes in distinguishing tumor cells from mononuclear phagocytic cells and from the sloughing mesothelial lining of the cavity. Cells floating free in fluid become very large and atypical. Multinucleate elements arise. Mitoses occur. Even alveolar structures may be encountered. Unless all these features are taken into account it is unsafe to hazard an opinion that the cells of an exudate are neoplastic. We have hesitated to make that diagnosis even when there was good reason to believe that tumor cells were widely implanted over a serous surface. Occasionally the relation of the cells to a bit of included fibrous stroma may be the decisive point in diagnosis, but such relations are not apt to be found when several liters of fluid are submitted for examination. Sectioned clots from centrifuged exudates are not any more satisfactory than simple smears from the centrifuged fluids.

Aspirations from the thyroid are difficult to interpret. We have examined 45 such specimens. It has been possible to detect the more atypical tumors, such as the giant and spindle cell carcinomas, but the differentiation of papillary or alveolar carcinomas of the thyroid from the more active and hyperplastic forms of Graves' disease, or even the atypical regenerative processes surrounding the colloid adenoma, has not been consistently possible. Struma lymphomatosa is readily confused with the small cell carcinoma of the thyroid, even in sections. Such being the case, it is unlikely that the two conditions can be diagnosed from smears.

Few attempts have been made to secure material from tumors of the abdominal viscera. The liver has been aspirated several times with success, so far as determining the presence of tumor is concerned, but not its actual type. The stomach has been aspirated for verification of tumor, usually at the time of surgical exploration. So far satisfactory material has been difficult to obtain. The kidney region has been needled several times but in each instance to rule out the presence of recurrences in or around nephrectomy scars. On one occasion only has it been possible to determine the type of tumor from the cell morphology.

Aspiration is very useful in distinguishing prostatic carcinoma

from benign lesions of the prostate.[3] Much the same problems are presented as in the case of breast tumors. The cells obtained are usually diffusely arranged and distinct alveolar characteristics are lacking. They are larger, disconnected and more atypical than the cells of benign hyperplasia. If tissue is obtained from the epithelium of the major prostatic ducts near their point of entrance into the urethra the appearance is suggestive of cells obtained from fibroadenoma of the breast. It may even suggest epidermoid carcinoma. We have learned to disregard these appearances in prostatic aspirations. To date there have been 104 aspirations of prostates.

Suspected embryonal carcinomas of the testes have been aspirated but 3 times. With the Ascheim-Zondek test so uniformly positive in testicular tumors one has a reliable source of confirmation of clinical diagnosis without subjecting these widely disseminating lesions to even the possible danger of the trauma of aspiration.

Aspiration of bone lesions has met with considerable success.[4] The principal indications for aspiration are to make differential diagnoses between the following types of lesions: (1) benign giant cell tumor and central osteogenic sarcoma; (2) endothelial myeloma, osteogenic sarcoma and osteomyelitis; (3) plasma cell myeloma and metastatic carcinoma; (4) osteogenic sarcoma and metastatic carcinoma. Of these problems the last is by far the most difficult, since a very cellular osteogenic sarcoma (Fig. 7) simulates closely metastatic cancer in smears, especially since the formation of bone or osteoid tissue is not apt to be ascertainable among the unattached cells that usually enter the needle. If cartilage is present it is detected more readily. It is obvious that a diagnosis of benign giant cell tumor may not always be correct, since the malignant osteolytic sarcoma may contain many epulis giant cells, yet the difficulties encountered have been fewer than might have been anticipated. The typical benign giant cell tumor yields large numbers of characteristic multinucleated giant cells that are unmistakable (Fig. 6). Furthermore it must be remembered that the central tumor most apt to be confused with giant cell tumor radiographically is usually of the large spindle cell fibrosarcoma variety, a type rather readily distinguished by aspiration.

Endothelial myeloma in smears shows enormous numbers of small, round or polyhedral cells, either diffusely arranged or in sheets. In some respects it may resemble the smear from a hyperplastic lymph

node. On three occasions we have been able by considerable search to detect the small rosettes characteristic of this tumor (Fig. 8). Osteomyelitis, of course, yields large numbers of inflammatory cells. Plasma cell myeloma is readily diagnosed by the appearance of large numbers of characteristic plasma cells with their typical nuclei and opaque cytoplasm. We have had no experience with aspirating the rarer varieties of bone tumors.

Of the soft part tumors the giant cell tumors of tendon origin are readily diagnosed by their content of epulis giant cells. These cells must be distinguished from the Langhans cells of tuberculous tenosynovitis. Neurosarcoma, for the most part, cannot be differentiated from myosarcoma or liposarcoma, and the harder, more fibrous types of neurosarcoma are apt to yield no cells when aspirated.

The diagnosis of non-pigmented melanoma has not been possible, except with the proper history. One should hesitate to draw any conclusion from the simple occurrence of brownish pigment without employing specific stains for iron, since the steel aspirating needle collects traces of rust, which simulates both blood pigment and melanin. With proper precautions pigmented melanoma can be readily diagnosed.

Summary and Conclusions

Needle aspiration is in our experience an expeditious, practical method of tumor diagnosis. In approximately 2500 cases we have observed no untoward result following its use. The interpretation of smears of aspirated material often requires competent clinical assistance. Diagnosis by aspiration is as reliable as the combined intelligence of the clinician and pathologist makes it. The pathologist who ventures to interpret the material obtained by aspiration will have to revise or relearn many criteria. The clinician must appreciate how far the pathologist can logically go in interpreting the smear. Both must maintain a sympathetic attitude toward a new procedure. It is safe to state that in our own institution the method has so established its usefulness that it has acquired a permanent place as a means of diagnosis.

REFERENCES

1. Martin, Hayes E., and Ellis, Edward B. Biopsy by needle puncture and aspiration. *Ann. Surg.*, 1930, **92**, 169–181.

2. Forkner, Claude Ellis. Material from nymph nodes of man. *Arch. Int. Med.*, 1927, **40**, 532–537, 647–660.

3. Ferguson, Russell S. Prostatic neoplasms: their diagnosis by needle puncture and aspiration. *Am. J. Surg.*, 1930, N. S. **9**, 507–511.

4. Coley, Bradley L., Sharp, George S., and Ellis, Edward B. Diagnosis of bone tumors by aspiration. *Am. J. Surg.*, 1931, N. S. **13**, 215–224.

DESCRIPTION OF PLATES

PLATE 128

FIG. 1. Mucin and cartilage cells aspirated from tumor of scalp.

FIG. 2. Small spindle cells embedded in mucin aspirated from a mixed tumor of the parotid.

FIG. 3. Larger, atypical, irregular hyperchromatic cells aspirated from a malignant submaxillary gland tumor. Same magnification used in Figs. 2 and 3.

FIG. 4. Coherent sheets of rather small, regularly arranged cells obtained by aspiration from a fibro-adenoma of the breast.

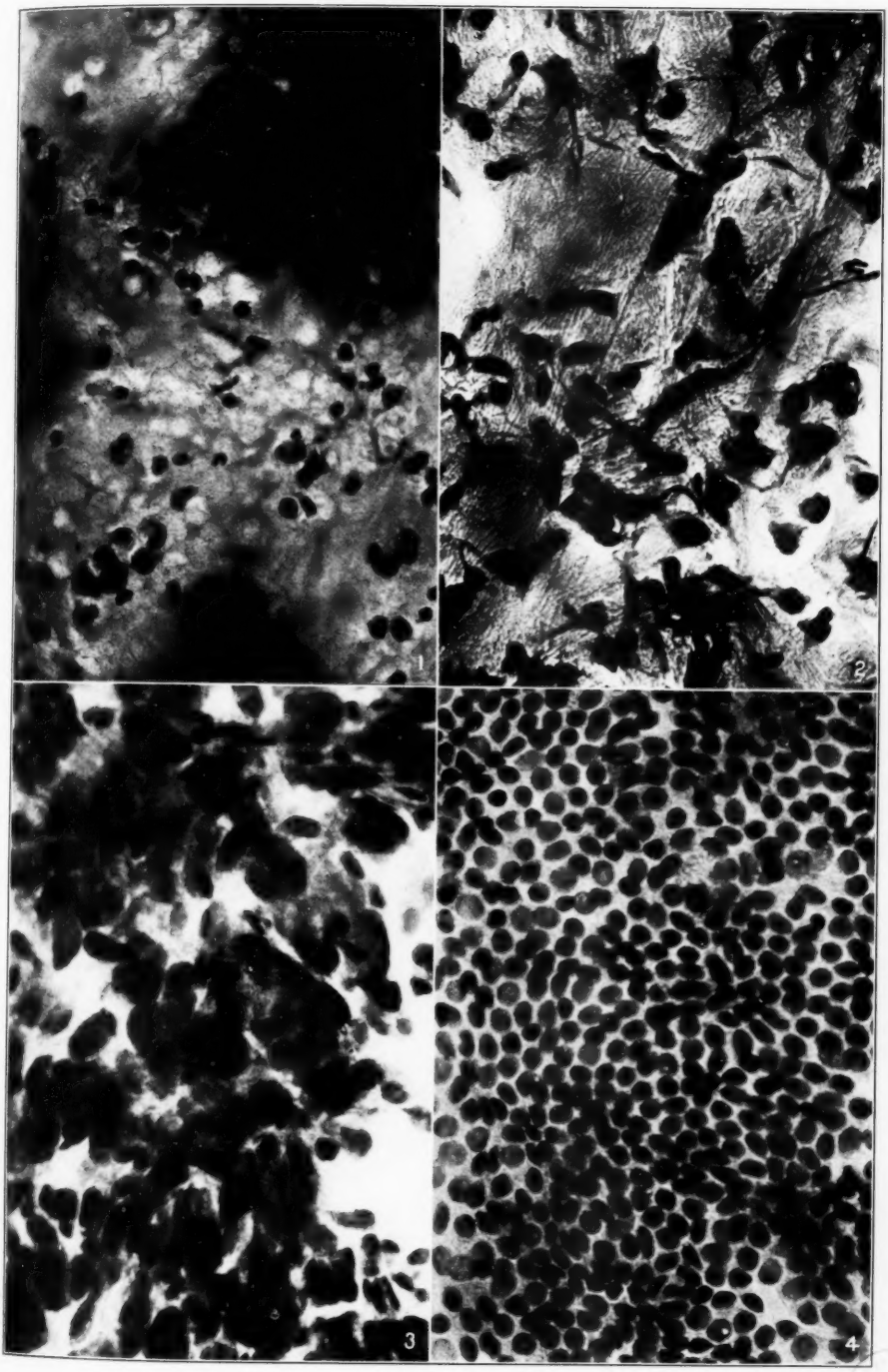


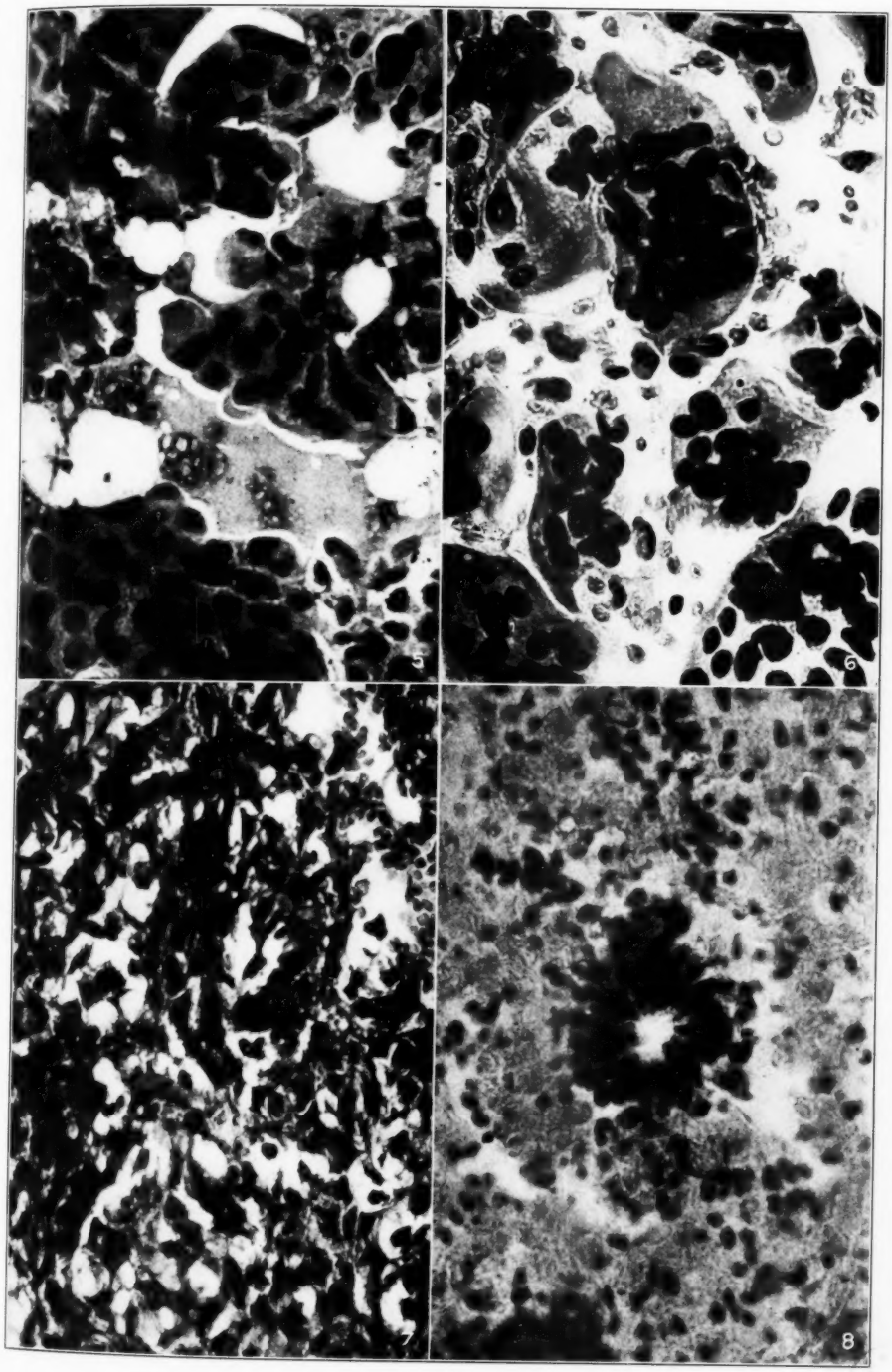
PLATE 129

FIG. 5. Mammary carcinoma. Same magnification used in Figs. 4 and 5.

FIG. 6. Benign giant cell tumor of bone. Numerous giant cells of the epulis type are seen.

FIG. 7. Closely packed, small spindle cells aspirated from an osteogenic sarcoma.

FIG. 8. Aspirated material from an endothelial myeloma. A typical rosette is seen. Figs. 6, 7, and 8 reproduced at same magnification for comparison.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THE PATHOLOGY OF THE BONE MARROW IN SPRUE ANEMIA*

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Introduction

The study of bone marrow by means of specimens removed during life has been applied frequently to forms of anemia other than those associated with tropical sprue. Ghedini,[1] Morris and Falconer,[2] and Zadek [3, 4] all studied the bone marrow of living patients with pernicious anemia. It remained for Peabody [5, 6] to apply more modern knowledge to the study of the finer structure of bone marrow. In his final paper he showed most clearly that in pernicious anemia the marrow was hyperplastic during relapse and tended to return to normal during remission. Although the existence of a relation between the macrocytic anemia of sprue and of pernicious anemia has become manifest, existing observations of the bone marrow in sprue with anemia are few in number and confusing. Ashford [7] briefly reported findings in specimens of tibial marrow studied during life by another worker. Both hyperplastic and aplastic histological pictures were found. No photographs are shown nor are any descriptions of the histological changes given. The author's conclusions as to the marrow pathology seem to be colored largely by existing hypotheses of marrow function based on the response of the peripheral blood to therapy. Mackie and Fairley [8] described the changes found mainly in the femoral and tibial marrows of a group of autopsied cases of sprue. They noted in certain instances hyperplastic marrow, such as is found in pernicious anemia. In others fatty marrow was found, with rare, circumscribed areas of activity. Still other marrows showed a type of gelatinous degeneration. These various mani-

* These observations were carried out in 1931 by the Commission of the Rockefeller Foundation for the Study of Anemia in Puerto Rico.

Received for publication June 1, 1933.

festations were considered to represent various stages, ranging from hyperplastic "active" marrow to atrophic "exhausted" marrow. The fact that the marrow of the long bones is constantly fatty in character under normal conditions and is the last to respond with hyperplasia was not emphasized. Their briefly reported histological studies added little to the descriptions of the gross picture as regards the character of the cellular reaction. However, the red femoral marrow of one patient was stated to have exhibited "production of numerous megaloblasts as well as nucleated red cells."

The most careful and detailed studies of the bone marrow in sprue are those reported by Krjukoff.[9] This worker studied biopsy material from the ribs of sixteen patients. He was impressed by the constancy with which he found a megaloblastic change of the marrow with the production of "lymphoid erythroblasts" of all sizes, megaloblasts and normoblasts. He does not mention any cases in which an inactive marrow was found. The uniformity of the pathological alterations found is in striking contrast to the observations of other investigators and conforms much better to what is now known to be the effect of adequate specific therapy.

The divergent results of these studies of sprue have, at least in part, been due to the fact that specimens from comparable areas of the marrow were not studied. Piney [10] has shown that only the ribs, flat bones, and vertebral column normally contain active cellular marrow in adult life. Peabody [5] has emphasized the fact that the marrow of a long bone, such as the tibia, is not necessarily homogeneous in structure and that the pathological process there does not necessarily correspond in extent and degree to that in the marrow of other bones. On the other hand, his own studies of pernicious anemia were carried out on specimens of tibial marrow because he felt that it was simpler in structure and hence presented alterations easier to interpret than the more complicated marrow of flat bones or sternum. After the clarification of the underlying cellular changes of the marrow in pernicious anemia as a result of Peabody's work, it appeared logical to us to examine a site of normally active marrow in sprue. It was felt that in this way early alterations might be observed more readily and conclusions drawn with more certainty as to the presence of hyperplasia or aplasia of the functional elements. For these reasons this communication is based largely upon examination of specimens of sternal bone marrow removed at operation.

Methods

Twenty-two patients with sprue anemia of a macrocytic type were studied. In certain instances specimens were removed both before and after remissions, in others during the height of the reticulocyte response to liver therapy. The eventual death of three patients made it possible to compare the postmortem pathological picture with that seen during life. In still another group abnormalities of the clinical course were correlated with the histopathological alterations of the marrow.

The procedure of removing the specimens of sternal marrow was carried out in the operating room under complete surgical precautions for asepsis. A longitudinal midline incision about 4 cm. long was made from the third to the fifth costal cartilages, and was extended down to the periosteum. Two incisions were made in the periosteum, each about 1 cm. long and at right angles to each other. The periosteum was carefully retracted to expose an area of bone about 1 cm. in diameter. With a small crown trephine the outer table of bone was removed as a button about 0.4 cm. in diameter. With a bone curette enough marrow was removed to ensure the inclusion in microscopic sections of actual marrow tissue. The bone button was replaced and hemorrhage from the bone stopped with bone wax. The periosteum was brought together with No. 0 plain catgut. One or two subcutaneous stitches of the same suture material were used to approximate the subcutaneous tissue and the skin was closed with interrupted silk sutures. A dry dressing applied with considerable pressure from heavy adhesive straps was kept in place for twenty-four hours.

The tissue was fixed immediately in Zenker's fluid. After the usual steps of dehydration the tissue was embedded in paraffin and cut at 6 microns without decalcification. Mallory's phosphotungstic acid hematoxylin, eosin-methylene blue and the Giemsa stain were employed. Certain blocks were cut serially.

Results

The results of the microscopic examination of the bone marrow are summarized in the table and will be discussed below. In addition a few illustrative cases are briefly cited, and the microscopic picture described in detail.

TABLE I

Table Showing Blood Picture and Bone Marrow Histology in Macrocytic Anemia of Sprue

Case No.	Blood picture: Red blood cells in millions	Hemoglobin *per cent*	White blood cells in thousands	Reticulocytes *per cent*	Mean corpuscular volume in cu. μ	Icterus index units	Color index	Bone marrow: Biopsy or postmortem	Bone source	General cellularity	Megaloblasts and erythroblasts	Normoblasts	Fat	Comment
								Untreated Cases						
1	2.15	54	8.1	1.8	130	2	1.25	Biopsy	Sternum	++	++	++	++	Very early though definite megaloblastic preponderance (Fig. 1).
2	1.76	49	6.2	1.4	126	5	1.39	"	"	+++	+++	+	+	Moderate megaloblastic proliferation and preponderance (Fig. 2).
3	1.41	40	3.3	3.2	126	4	1.42	"	"	++++	++++	–	–	Intense megaloblastic proliferation and preponderance (Fig. 3).
4	1.42	48	2.2	6.6	140	40	1.69	"	"	++++	++++	–	–	" " " " "
5	0.98	28	2.2	0.8	143	25	1.43	"	"	++++	++++	–	–	" " " " "
6	2.51	55	3.3	4.8	101	2	1.10	"	"	++++	++++	–	–	" " " " "
7	1.96	46	9.8	1.4	114	2	1.17	"	"	++++	++++	–	–	" " " " "
8	1.92	42	4.8	2.0	102	5	1.10	"	"	++++	++++	–	–	" " " " "
9	2.67	66	7.4	2.2	119	3	1.24	"	"	++++	++++	–	–	" " " " "
10	1.15	34	5.1	3.8	131	30	1.48	"	"	++++	++++	–	–	" " " " "
11	2.52	63	8.1	3.2	128	3	1.25	"	"	++++	++++	–	–	" " " " "
12	3.12	73	7.5	1.6	107	2	1.14	"	"	++++	++++	–	–	" " " " "
13	1.25	29	1.8	1.0	104	18	1.16	"	"	++++	++++	–	–	" " " " "
14	1.68	55	5.0	4.6	134	5	1.63	"	"	++++	++++	–	–	" " " " "
15	2.40	54	1.9	0.4	113	40	1.12	"	"	++++	++++	–	–	" " " " "
16	1.46	40	3.0	0.1	121	5	1.37	"	"	+++	++	+	+	Moderate megaloblastic proliferation and preponderance.

17	1.05	29	2.9	8.8	157	12	1.38	Postmortem	Sternum and femur	++++	++++	–	–	Phagocytosis of red cells present (Fig. 6).
18	2.86	68	7.6	1.6	119	3	1.19	"	"	++++	++++	–	–	Sternal marrow red, femoral partly red. Lung abscess. Microscopically very loose structure.
19	0.97	19	7.3	4.5	103	30	0.98	"	"	++++	++++	–	–	Brick red marrow sternum and upper third of femur. Transfusion. Microscopic structure confused. Phagocyted erythrocytes.

Treated Cases

20	0.94	19	3.6	4.4	115	10	1.00	1st biopsy	Sternum	++++	++++	+	–	Before remission. Many megaloblasts, few normoblasts (Fig. 4), almost no fat cells.
20	2.97	60	3.3	4.2	100	2	1.01	2nd biopsy	"	++	+	+++	++	20 days after intramuscular liver extract. Predominant normoblasts (Fig. 5).
21	0.83	26	3.0	14.0	162	3	1.57	Biopsy	"	++	++	+	+	9th day oral liver extract. During reticulocyte rise. Megaloblasts separating into clumps. Normoblasts appearing.
22	1.40	29	2.3	21.6	114	6	1.04	1st biopsy	"	+++	++	++	+	9 days after liver extract intramuscularly. During reticulocyte rise. Decrease in megaloblasts. Increased normoblasts.
22	2.69	55	2.8	2.0	99	3	1.02	2nd biopsy	"	+	+	++++	+++	30 days after liver extract. Late remission. Marrow picture approaches normal, except for increased normoblasts.

Cellular Composition of the Sternal Bone Marrow in Untreated Cases of Sprue

CASE 1. *Clinical History:* The patient was a Puerto Rican housewife 47 years of age. There was a history of fairly typical sprue for one year, with loss of weight, gastro-intestinal disturbance, glossitis and anemia. Before the biopsy the patient had been treated for sixteen days with autolyzed yeast, to which she showed a slight reticulocyte response and slight clinical improvement. By the time of the biopsy the lingual and gastro-intestinal symptoms were somewhat improved without significant changes in blood values.

On the day of the biopsy, Aug. 26, 1931, examination of the blood showed: Red blood cells 2,150,000 per cmm.; hemoglobin 54 per cent (Sahli); color index 1.25; mean corpuscular volume 120 cu. μ; leukocytes 8100 per cmm.; reticulocytes 1.8 per cent; icterus index 2. The blood smear was consistent in appearance with the macrocytic anemia of sprue.

Bone Marrow Biopsy: The tissue appeared only moderately cellular and contained quite a large number of bone spicules. It was red-brown in color. The microscopic appearance is illustrated in Figure 1. A considerable amount of fat is present. The number of cells is distinctly greater than that observed in normal sternal marrow. Groups of from six to eight megaloblasts are seen with round nuclei containing rather heavy masses of chromatin and occasionally in mitosis. Certain cells of this general type are larger, with a considerable amount of rather pale, basophilic, cytoplasm. The latter is the type of cell that is present in such large numbers in the bone marrow in pernicious anemia in relapse. Smaller cells, diffusely scattered, with round nuclei containing dense masses of chromatin are numerous. These have a heavily basophilic cytoplasm that varies greatly in amount, though it is never so great as in the megaloblast. These cells are considered to be erythroblasts of varying degrees of maturity. A considerable number of normoblasts is present, many more than are seen in the marrow of pernicious anemia in relapse or in marrows from patients with sprue of a more severe degree. Megakaryocytes are present in about normal numbers, as are cells of the granulocytic series. Many sinusoids are closed.

Comment: Subsequent heavy dosage with liver extract administered intramuscularly failed to effect a reticulocyte rise or improvement in blood values. When ferric ammonium citrate was administered, however, a distinct reticulocyte rise appeared and subsequently a return to normal blood levels resulted. In this marrow the frequent occurrence of normoblasts suggests a deficiency of iron

as well as of liver extract, which is confirmed by the fact that both liver extract and iron were required to bring about improvement. In contrast to the findings in the bone marrow of Case 2, the histological changes are slight. It is conceivable that this is the earliest change, since the illness of the patient was short, and the blood values were at a relatively high level.

CASE 2. *Clinical History:* The patient was a male journalist 60 years of age, who had suffered from gastro-intestinal disturbance, glossitis, and weakness for ten years. For the ten days preceding the biopsy the patient was treated with 6 gm. of ferric ammonium citrate daily without effect.

On the day of the biopsy, Sept. 8, 1931, the blood examination showed: Red blood cells 1,760,000 per cmm.; hemoglobin 49 per cent (Sahli); color index 1.39; mean corpuscular volume 126 cu. μ; white cells 6200; reticulocytes 1.4 per cent; icterus index 5. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: The marrow did not appear remarkable at operation. Some increase in cellular over bony tissue was present and the color was distinctly reddish. The microscopic appearance is illustrated in Figure 2. A moderate amount of fat is present, up to thirty cells per high power field. Between the fat cells are masses of cellular tissue. The vast majority of the cells present are megaloblasts, some arranged in groups and others scattered diffusely throughout. They are larger, in general, than are those seen in Case 1 and have considerably more cytoplasm. Mitoses are frequent. Many erythroblasts of varying degrees of maturity are seen. The relative number of normoblasts is strikingly decreased. A few cells of the granulocytic series are present. The marrow is similar to that seen in pernicious anemia in relapse, except for the lack of complete replacement of fat by cellular tissue. The sinusoids are open and contain many adult red cells. The uniformity of the cellular picture is most striking. Megakaryocytes are sharply reduced in number.

Comment: The administration of ferric ammonium citrate effected no improvement. Liver extract given orally brought about a slight reticulocyte rise and by subsequent parenteral administration restoration of normal blood values was slowly attained. This result could perhaps have been prognosticated from the changes present in the marrow. That the response was a slow one might be associated with the fact that although the cell type was uniform, complete replacement of fat had not taken place. This marrow is intermediate in

type between that of Case 1, where megaloblastic activity was mild and early, and much fat present, and that of Case 3, in which a picture quite similar in all respects to that of pernicious anemia was seen.

CASE 3. *Clinical History:* The patient was a Puerto Rican housewife, 60 years of age, who had suffered from malnutrition for one year, and during the past three months from glossitis, diarrhea, and edema of both feet. There had been considerable loss of weight.

On the day of the biopsy, Aug. 26, 1931, the blood examination was as follows: Red blood cells 1,410,000 per cmm.; hemoglobin 40 per cent (Sahli); color index 1.4; mean corpuscular volume 125 cu. μ; white blood cells 3300 per cmm.; reticulocytes 3.2 per cent; icterus index 4. The blood smear was typical of the macrocytic anemia of sprue.

Bone Marrow Biopsy: Grossly the marrow appeared dark red and hyperplastic. Definite replacement of bone spicules had taken place. Little fat was seen. As shown in Figure 3, microscopic examination bears out this impression. Almost no fat is present, averaging fewer than one fat cell per high power field. The tissue is rather vascular and extremely cellular. Masses of large megaloblasts are present. These cells are somewhat irregular in size and outline. The nuclei are rounded or oval and contain rather little chromatin, that present being clumped in irregular masses. The cytoplasm is slightly basophilic and irregular in outline. Smaller clumps of cells of somewhat different character are seen. These cells have round nuclei with heavy masses of chromatin. The cytoplasm varies in amount and is deeply basophilic. These cells are considered to be erythroblasts. Very few normoblasts are seen. A moderate number of megakaryocytes is present, as is some myelopoiesis with all stages in the development of the granulocytic cell series.

Comment: The picture is one of diffuse megaloblastic hyperplasia, similar in nature to that seen in pernicious anemia in relapse. As might be expected with the type of bone marrow change described, a prompt reticulocyte response followed the intramuscular injection of liver extract. This response was followed by eventual restoration of normal blood values.

In the three cases just described different degrees of the same fundamental histological change have been observed. The presence of such definite variations in the intensity of the process, even in the sternal marrow, is considered to be a partial explanation of the failure of many investigators to find active marrow in the long bones of

certain autopsied cases of sprue anemia. Presumably, in such instances, the pathological change has failed to extend to the long bones, though strikingly present in the sites of most active blood formation where the earliest changes would be expected to appear. In no case examined, and many, as may be seen from the table, had anemia of an extreme degree, was the sternal bone marrow atrophic. This is in agreement with the observations of Krjukoff and quite opposed to the statements of those who have confined their studies to the marrow of the long bones.

The Effect of Therapy on the Cellular Composition of the Sternal Bone Marrow in Sprue

Hitherto the marrow in cases of sprue anemia has not been studied before and after treatment capable of producing reticulocyte rises and restoration of normal blood values. Two cases of the present series were subjected to sternal punctures at different stages of the disease and the histological pictures compared. The changes were similar in both cases.

CASE 20. *Clinical History:* The patient was a male Puerto Rican, 35 years of age, who entered the hospital complaining of weakness of six months duration. For four months he had suffered from watery diarrhea and glossitis. The day before the first biopsy he received a transfusion of 200 cc. of whole blood and an injection of an effective extract derived from 100 gm. of liver.

On the day of the first biopsy, Aug. 6, 1931, a blood examination showed: Red blood cells 940,000 per cmm.; hemoglobin 19 per cent (Sahli); color index 1.0; mean corpuscular volume 115 cu. μ; white blood cells 3600 per cmm.; reticulocytes 4.4 per cent; icterus index 10.

First Bone Marrow Biopsy: In the gross the tissue was soft, deep red, and had definitely replaced a large part of the bony trabeculae. Histologically, as shown in Figure 4, all resemblance to normal marrow structure is lost. No fat is present. The tissue is an almost solid mass of large, pale cells, with nuclei containing scattered masses of chromatin. Their outline is irregular, the cytoplasm moderately basophilic, and the cells are in close apposition. These cells are considered to be megaloblasts. Scattered diffusely throughout in large numbers are somewhat smaller cells with round nuclei containing dense and heavy chromatin. The cytoplasm of these cells is small in amount and is somewhat more deeply basophilic. Many mitoses are present. Nucleated red cells are almost entirely absent. Myelo-

poiesis is slight. Only a limited number of megakaryocytes are present. The vascular channels are obscured by the enormous cellular overgrowth. The whole picture is quite consistent with that seen in severe pernicious anemia.

The effect of liver extract on this patient was entirely similar to that occurring in Addisonian pernicious anemia. In response to the single injection of liver extract derived from 100 gm. of liver the reticulocytes reached a peak of 38 per cent on the fifth day and the blood values rose rapidly, as similar therapy at ten day intervals was maintained.

Twenty days after the first biopsy and after remission had occurred, though complete restoration of the blood levels to normal had not taken place, a second biopsy was performed. On the day of the second biopsy, Aug. 26, 1931, the blood examination showed: Red blood cells 2,970,000 per cmm.; hemoglobin 60 per cent (Sahli); color index 1.02; mean corpuscular volume 100 cu.μ; white blood cells 3300 per cmm.; reticulocytes 4.2 per cent; icterus index 2.

Second Bone Marrow Biopsy: Histologically, the picture is quite different from that of the first biopsy, as shown by a comparison of Figure 4 with Figure 5. Fat cells are present up to ten to twelve per high power field. A few large pale megaloblasts with irregular nuclei are present in groups of three or four. The solid background of these cells seen in the first specimen is completely replaced by sheets of normoblasts that greatly outnumber any other cell type present. Vascular channels are open, myelopoiesis is proceeding, and a fair number of giant cells may be seen. Clearly, the administration of effective therapy caused a maturation of megaloblasts to normoblasts with the production of a bone marrow morphology more nearly approaching the normal and so resembling the transition observed during the treatment of pernicious anemia.

Phagocytosis of Erythrocytes by Bone Marrow Cells in Sprue

The presence of erythrocytes in the cytoplasm of large cells of the bone marrow in pernicious anemia has been described frequently in specimens of marrow removed at autopsy. At a time when current views associated pernicious anemia with increased blood destruction, based upon the observations of the increased serum bilirubin content and an output of bile pigment greater than normal in the

excreta, Peabody considered that phagocytosis might be of significance in the production of the anemia. In an early paper Peabody and Broun [11] gave a detailed description of the phagocytic picture in pernicious anemia, as seen in tissue fixed postmortem, and compared it with postmortem material from other types of disease. Although phagocytosis occurred, especially in infectious diseases, it was apparently greater in cases of pernicious anemia where death occurred in an acute stage. Later observations of biopsy material, however, failed to reveal erythrocytes in the cytoplasm of large marrow cells and led to the conclusion that the cytological appearances, which were supposed to indicate the phagocytosis of red blood cells during life, were really postmortem, or at least terminal phenomena.

Study of postmortem specimens of bone marrow from two cases of sprue anemia in Puerto Rico revealed the same phenomenon, so frequently observed in marrows of patients dying of pernicious anemia. In both instances erythrocytes are seen in the cytoplasm of large bone marrow cells, as shown in Figure 6. In both of these specimens the architecture of the marrow is seriously distorted, as compared with tissue removed at biopsy. In the latter, closely packed masses of cells, uniform in size and shape and easily identifiable, with well marked vascular channels and stroma structure are seen. In the postmortem specimens the structure is extremely loose and almost unrecognizable. There is an enormous variation in size and shape of the cells. Some are very large and have an irregular, slightly basophilic cytoplasm, which contains large numbers of erythrocytes. Since this was not observed in any of the specimens removed at biopsy the conclusion is unavoidable that it is a postmortem change. In neither case was the body kept more than eight hours before the tissue was fixed, although lack of facilities for refrigeration and the heat of the climate may have accelerated a postmortem alteration. Peabody states that the cells that ingest erythrocytes in the bone marrow of pernicious anemia are clasmatocytes. He quotes the observation of Rich [12] that clasmatocytes may ingest red cells when grown *in vitro*. From the observations here presented no conclusion as to the nature of the phagocytic cell can be drawn. Only further evidence, based on supravital and tissue culture study of material removed at autopsy, can be expected to solve the problem.

Discussion

The material presented indicates that, as concerns the fundamental histopathological picture of the bone marrow during exacerbation, during remission and at postmortem, the changes accompanying the macrocytic anemia of tropical sprue are similar to those found in pernicious anemia, as described by others.

Peabody confirmed the previous observation of Zadek that during remission the bone marrow became less cellular; and for the first time accurately described the microscopic appearances both during remission and in relapse. He considered the hyperplasia occurring in relapse to be an extensive proliferation of primitive marrow cells, chiefly megaloblasts, associated with a relative decrease in the other elements, including the fat cells. Remission was shown to be characterized by a picture more nearly normal, with few megaloblasts, a relative increase of normoblasts and mature red blood cells, and the reappearance of fat. The anemia of relapse was explained by the functional ineffectiveness of the marrow resulting from the failure of the megaloblasts to form mature erythrocytes. In the sternal marrow of nineteen patients with untreated sprue the same fundamental pathological change was found, an increase in number and size of the megaloblasts, a decrease in the amount of fat present and in the number of megakaryocytes and cells of the granulocyte series. The number of normoblasts was strikingly small in comparison to the number of megaloblasts. The differences in the bone marrow changes reported by previous observers can thus apparently be explained by the fact that only the marrow of the long bones was studied. Beyond question the extent of marrow involvement is greater in pernicious anemia than in certain cases of sprue, but the changes occurring in the normally active marrow of the adult, obtained here by sternal biopsy, present a uniform picture.

Furthermore, the effect of specific therapy in sprue anemia is folfowed by the same maturation of megaloblasts to normoblasts and restoration of normal morphology of the marrow, as occurs in pernicious anemia. This fact parallels the similarity of the blood changes in the two conditions. Although, as a rule, the effect of liver extract on the lingual and gastro-intestinal symptoms is as striking as in pernicious anemia, the hematopoietic response is frequently not so marked for a given blood level in sprue as in per-

nicious anemia. This is probably to be explained, at least partly, on the basis of the fact that in pernicious anemia the megaloblastic hyperplasia involves a greater amount of the normally inactive bone marrow. It is also clear that in sprue a combination of therapy with iron, as well as with liver extract, is necessary in many cases, a fact that correlates with the findings in the bone marrow of certain patients, as illustrated by Case 1. Despite this fact, the basic similarity of the blood pictures and of the changes in the active portions of the bone marrow in relapse and in response to similar therapy would seem to be strong evidence for a similar etiological mechanism. Observations have been made by Castle and Rhoads [13] that indicate the macrocytic anemia of sprue is mainly the result of a deficiency similar to that existing in pernicious anemia, although brought about in a somewhat different manner.

Conclusions

1. Observations on the bone marrow of sprue anemia made on tissue obtained at biopsy in different stages of the disease show that the changes are similar to those of pernicious anemia.

2. During relapse the essential change is a proliferation of megaloblasts and suppression of maturation to the normoblast stage.

3. During remission the marrow tends to return to normal with a great increase in the number of normoblasts and mature red cells in the marrow.

4. Phagocyted erythrocytes were observed in the bone marrows removed at autopsy but not in those removed during life.

The authors wish to acknowledge their indebtedness to Drs. W. R. Galbraith, George C. Payne, and to Herman A. Lawson for their cooperation in the conduct of these observations, as well as to Mr. Emil Bohnel and Mr. Louis Zetzel for their assistance in making the blood studies.

REFERENCES

1. Ghedini, G. Neue Beiträge zur Diagnostik der Krankheiten der hämatopoetischen Organe mittels Probepunktion des Knochenmarks. *Wien. klin. Wchnschr.*, 1910, **23**, 1840–1847.

2. Morris, L. M., and Falconer, E. H. Intravitam bone marrow studies. Part II. Survey of the clinical field. *Arch. Int. Med.*, 1922, **30**, 490–506.

3. Zadek, I. Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Schweiz. med. Wchnschr.*, 1921, **51**, 1087–1091.

4. Zadek, I. Blut- und Knochenmarkbefunde am Lebenden bei kryptogenetischer perniciöser Anämie, insbesondere im Stadium der Remission. *Ztschr. f. klin. Med.*, 1922, **95**, 66–87.

5. Peabody, F. W. A study of hyperplasia of the bone marrow in man. *Am. J. Path.*, 1926, **2**, 487–502.

6. Peabody, F. W. The pathology of the bone marrow in pernicious anemia. *Am. J. Path.*, 1927, **3**, 179–202.

7. Ashford, B. K. The differential diagnosis of sprue and pernicious anemia. *Am. J. Trop. Med.*, 1932, **12**, 199–215.

8. Mackie, F. P., and Fairley, N. H. The morbid anatomy of sprue. *Indian J. M. Research*, 1928–29, **16**, 799–811.

9. Krjukoff, A. Anämie bei Sprue. *Folia hæmat.*, 1928, **35**, 329–352.

10. Piney, A. The anatomy of the bone marrow: With special reference to the distribution of the red marrow. *Brit. M. J.*, 1922, **2**, 792–795.

11. Peabody, F. W., and Broun, G. O. Phagocytosis of erythrocytes in the bone marrow, with special reference to pernicious anemia. *Am. J. Path.*, 1925, **1**, 169–183.

12. Rich, A. R. The formation of bile pigment from haemoglobin in tissue cultures. *Bull. Johns Hopkins Hosp.*, 1924, **35**, 415–416.

13. Castle, W. B., and Rhoads, C. P. Observations on the aetiology and treatment of sprue in Puerto Rico. *Tr. Assn. Am. Phys.*, 1932, **47**, 245–247. Abstr. *Lancet*, 1932, **1**, 1198–1199.

DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Case 1. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue of moderate degree and duration before remission produced subsequently only after addition of iron to liver extract therapy. Note presence of much fat, numerous normoblasts and moderate numbers of megaloblasts. Giemsa stain. × 1500.

FIG. 2. Case 2. Sternal marrow removed at biopsy from patient with macrocytic anemia of sprue. Note presence of fat and moderate megaloblastic preponderance. Cells of the myeloid series are present in considerable numbers. Giemsa stain. × 1000.

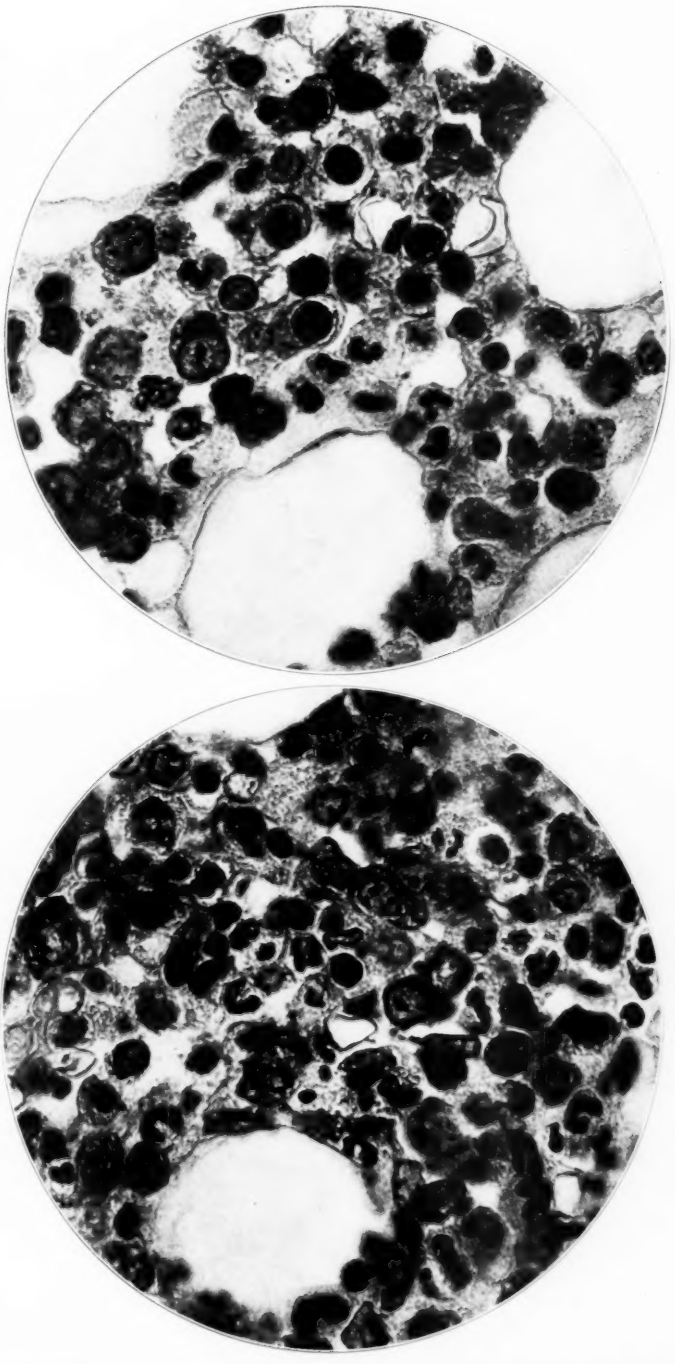

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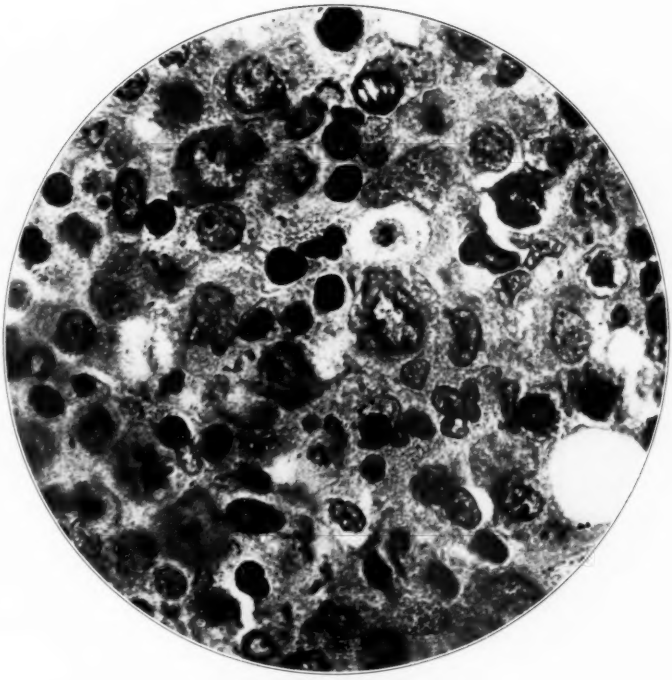
PLATE 131

FIG. 3. Case 3. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue. Note almost total absence of fat and intense megaloblastic proliferation with relatively few normoblasts and cells of myeloid series. Giemsa stain. × 1500.

FIG. 4. Case 20. Sternal marrow removed at biopsy from patient with severe macrocytic anemia of sprue before typical remission produced with liver extract. Note absence of fat with increased cellularity due largely to intense megaloblastic proliferation with occasional mitotic figures. Giemsa stain. × 1000.



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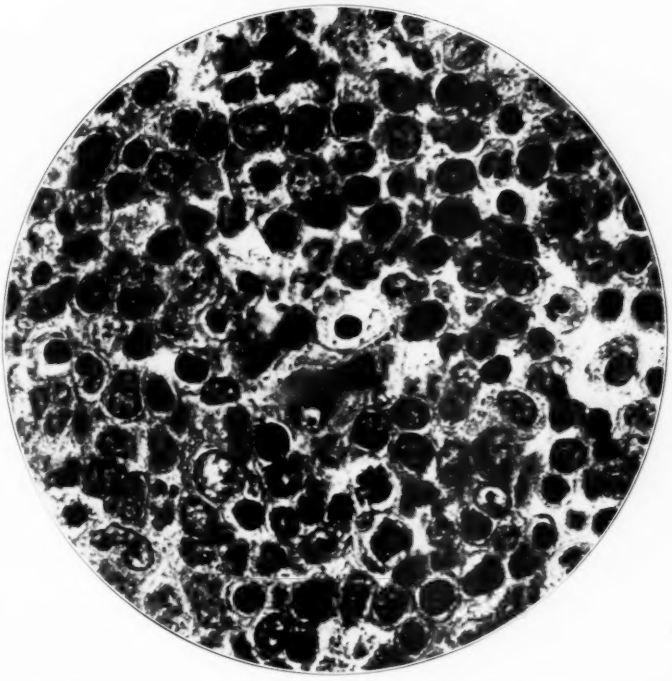

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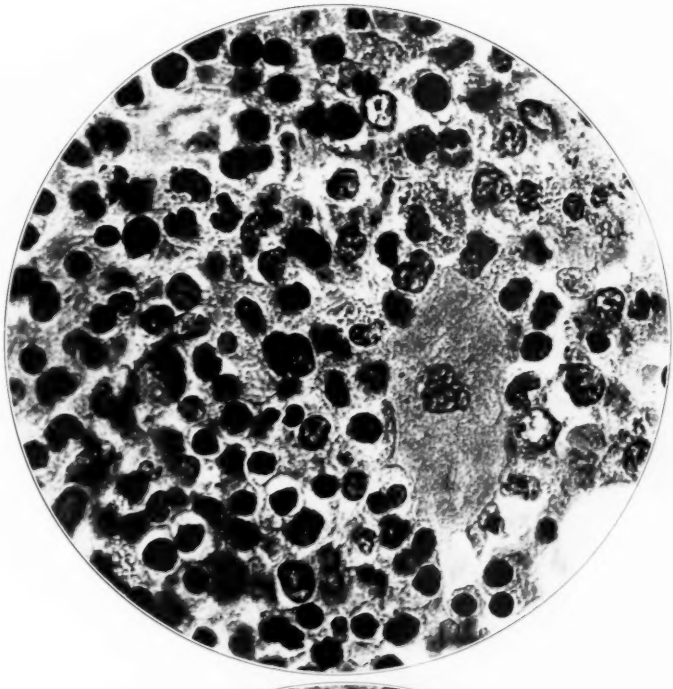
PLATE 132

FIG. 5. Case 20. Second sternal marrow removed at biopsy twenty days after injection of active liver extract, which produced a typical reticulocyte crisis. Note the decreased number of megaloblasts and the normoblastic preponderance. Giemsa stain. × 1000.

FIG. 6. Case 17. Sternal marrow removed at autopsy from patient dying of sprue with severe macrocytic anemia. Note loss of structural details and presence of phagocyted erythrocytes. Giemsa stain. × 1000.



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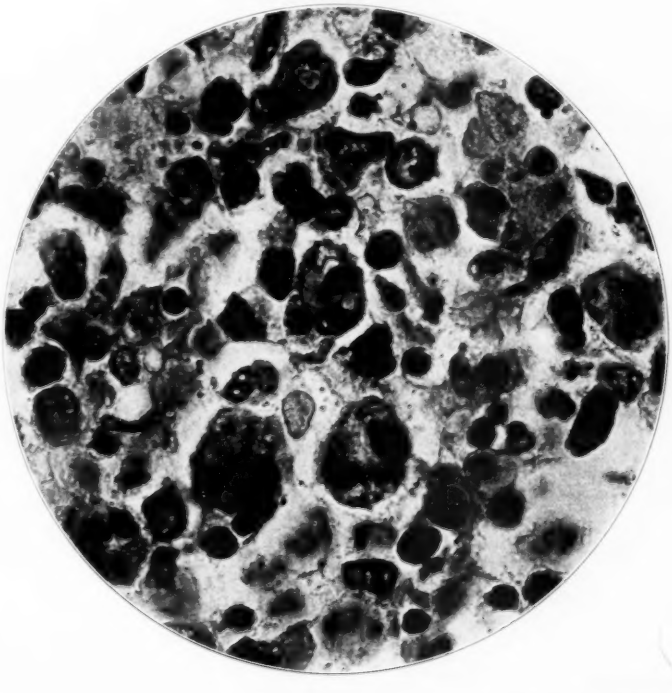

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